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Sexual maturation and reproductive parameters of wild and domestic stocks of white sturgeon, *Acipenser transmontanus*

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Sexual Maturation And Reproductive Parameters of Wild And Domestic Stocks Of White Sturgeon, *Acipenser transmontanus*

By

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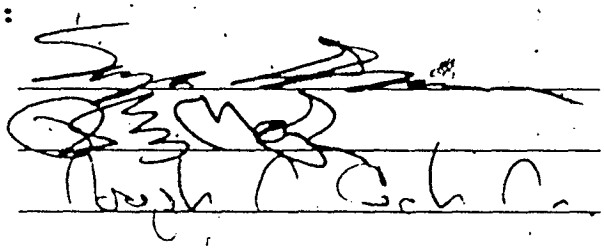

Committee in Charge

1989

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A special appreciation is for those who presented me with an amalgam of enlightening and gratifying ideas. They are now responsible for development of many of my concepts and opinions.

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INTRODUCTION

Sturgeons, largely because of their economic importance and decline of natural populations, have recently received attention from reproductive physiologists. There is extensive descriptive literature, primarily of Soviet origin, dealing with sturgeon reproductive patterns, endocrine glands, and microanatomy of reproductive organs (Barannikova 1987; Detlaf et al. 1981; Detlaf and Vassetzky 1988; Persov 1975; Raikova 1976). In regards with functions, this literature is mostly concerned with description of final oocyte maturation stages and induced spawning techniques. The chronology and physiological regulation of the gametogenic cycle remains poorly understood (Goyette et al. 1988; Barannikova and Fadeeva 1972; Trusov 1975, 1971). Our investigation focuses on gametogenesis and sexual maturation in white sturgeon, Acipenser transmontanus. Emphasis is placed in describing the ovarian cycle in females of wild stock, and monitoring of sexual development in domestically (i.e. hatchery) raised sturgeon. Because sturgeons differ from most other fishes in requiring a long time to reach sexual maturity and having a longer-than-annual ovarian cycle, understanding gametogenesis is important for the management of wild and cultured stocks and to provide the basis for breeding sturgeon species in captivity. Thus we also studied the normal function of the reproductive system as well as its

modification by pharmacological techniques in relation to broodstock development for aquaculture. Acipenser transmontanus is an anadromous sturgeon endemic to the Pacific estuaries of North America. Until recently, little information was available on its natural history (Lane 1985 and Scott & Crossman 1973), and on its reproductive cycle (Kohlhorst 1976; Goyette et al. 1988; Pycha 1956). In San Francisco Bay and adjacent Sacramento-San Joaquin estuary, mature individuals spend most of their life in the Bay, and move into the Sacramento River system in the late winter or early spring on their natural spawning migration (Kohlhorst 1976; Stevens & Miller 1970; Pycha 1956). Individuals are believed to mature between 10 to 20 years of age and have spawning intervals of 2 or more years (Kohlhorst 1980; Miller 1972; Pycha 1956; Semakula & Larkin 1968; Seymour 1968).

At the University of California Davis campus, a decade of sturgeon research has resulted in an important background on the biology and culture of white sturgeon. Most important has been the development of hatchery technology for production of white sturgeon fingerlings for fisheries restoration and commercial aquaculture (Conte et al. 1988; Doroshov 1985; Doroshov et al. 1983). Most recently, attempts to domesticate this species at the University and some private farms, also indicated that rearing for sturgeon flesh and possibly caviar may be feasible. Although this hatchery technology is now available, the reproduction of

sturgeon broodstocks in captivity has not been accomplished, and the functional side of gametogenesis in white sturgeon has not been investigated in depth. The lack of understanding of this complex nature of sturgeon reproduction has not allowed to support or formulate a fishery management plan or reliable seed production facility. Progress in the knowledge of sturgeon gametogenesis is, therefore, essential. To manage or develop sturgeon broodstocks we must be in full control of their reproduction.

MATERIALS AND METHODS

Fish and Holding Facilities

This project utilized wild stock and six year classes of domestically raised sturgeon. Wild fish were caught in San Francisco Bay, California, during the late fall and winters of 1983-1986. Fish were snagged by hooks on feeding grounds, brought alive onto a vessel and sampled for maturity. The description and illustrations of capture and handling procedures are provided by Cuanang (1984). Immature fish were released after taking biopsy samples of gonad tissue and blood samples. Selected ripe broodstock were acclimatized to fresh-water, transported in tanks to campus and induced to spawn at the UC Davis aquaculture facilities. Guidelines for sturgeon collection, maintenance, induce spawning, hatchery, and larval rearing followed those previously described by Doroshov et al. 1983 and Conte et al. 1988. As a result of spawning wild fish every year from 1980 through 1986, we established 6 year classes (age groups) of domestically raised F1 white sturgeon for development of captive broodstock. Fish were reared under natural photoperiod in 3.6 m and 6 m diameter round fiberglass tanks. Tanks were supplied with aerated underground and local irrigation water (50 l/min), with seasonal fluctuation of temperatures from 10-12°C in January to 18-20°C in July & August. Fish received artificial salmonid diets from their birth. Juveniles were initially

fed a Biodiet starter (Biodiet, Inc.; Warrenton, Oregon) and then switched to a dry trout pellets at feeding rates ranging from 10% (Age 0+) to 0.6% (Age 4+) of body weight per day. Feed was distributed through automatic and demand feeders.

Similar sampling protocols were followed for all wild and domestic stocks. The samples collected included: total and fork length (measured to the nearest 0.1 cm), body (and carcass when available) weight (weighed to the nearest 0.1 Kg), gonad weight, gonadal tissue for histological analysis and blood plasma. In wild stock age was determined using a section of the pectoral fin ray and following the techniques described by Cuerrier (1951) and Roussow (1957). Wild fish were sampled on site of capture and in the hatchery (ripe broodstock), while domestic animals were monitored periodically in the hatchery. Each year-class, except 1980, was divided into two groups. One group was used for necropsy sampling while the second group was raised for potential spawning, and used for biopsy sampling and hormone implantations. The two groups were held in separate tanks but reared under similar conditions. Fish for necropsy were sampled quarterly (January, April, July, and October) while those for biopsy in May and November. These fish were individually marked. Condition factor (K) and gonadosomatic index (G.S.I.) were calculated with these formulas:

$$K = (\text{Body weight in Kg}/\text{length in cm}^3) \times 100$$

$$GSI = (\text{Gonad weight}/\text{Body weight}) \times 100$$

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Growth rates were estimated using, polynomial equations and the instantaneous rate of growth equation used by Ricker (1979). The parameters were defined as:

IGR= ln W2 - ln W1/t2 - t1

Where IGR= instantaneous growth rate, ln= natural log of body weight in Kg, t= initial and final time interval in years. Other values, e.g. length-weight relationships, were made using standard fishery techniques following the procedures outlined by Bagenal (1978).

Animal Restraint and Fish Handling

Sampling of wild fish was done immediately after capture, without anesthesia. Fish were removed from the holding tanks and laid ventral side up on a canvas stretcher supported by two wooden sawhorses. Gill respiration was maintained by supplying each animal with ambient salt or fresh water delivered through a hose placed above its mouth. Blood was sampled from caudal vessels with vacutainers and gonadal tissue through abdominal incision (see below). Fish were transferred to an anesthetic bath containing 150-250 mg/l of MS-222. After each fish had been anesthetized they were weighed and placed on the stretcher. When necessary, anesthesia was applied by perfusing the gills with recirculating, aerated water containing 75 mg/l of MS-222. The anesthetic solution was delivered from a sump tank through a 1.5 cm I.D. water tubing inserted in the mouth of the fish. The recirculating sump consisted of a 30 l ice chest and 1/4 h.p. submersible pump. In the sump tank the

anesthetic bath was replaced regularly to prevent water quality deterioration. An additional fresh-water source was supplied to aid in the control and recovery of anesthetized animals. To minimize handling stress and sampling variability all animals were handled in the cool mornings, and starved for at least 24 hrs. before sampling.

Sex Determination and Surgical Procedures

Like other sturgeon species, white sturgeon do not exhibit external secondary sex characters, therefore, sex must be determined by visual examination of gonads and histological slides prepared from biopsy samples. Standard aseptic techniques were practiced when handling the biopsy equipment and fish. The surgical site and surrounding area was rinsed with 40% nitrofurazone, and a 0.5- 1.5 cm incision through the body wall was made with a scalpel. The site selected for biopsy was located anterior to the base of the pelvic fins (approximately three ventral scutes) and 1-2 cm lateral to the mid-ventral line. This area allowed easy access to the gonads, while reducing the possibility of traumatizing vital organs (e.g. liver and intestine). An Allis tissue forceps was carefully manipulated dorsally and laterally along the body wall until the gonads were located. A small biopsy sample (3-5 mm) was then cut by with the forceps or scalpel. Animals approaching maturity have distended gonads and were easily sampled. The abdominal incision was closed with one or two cross sutures, using a surgery pack 1-0 or 00 non-absorbable suture and chromic gut.

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with swaged half circle reverse cutting needle. The body surface of the fish was kept moist at all times and, following the operation, was washed with nitrofurazone. Depending on water temperature, small incisions were closed in a few days; 20-30 days were required to heal.

Tissue Preparation and Laboratory Analyses of Samples

Blood was taken by caudal puncture into 5-10 ml heparinized vacutainers equipped with 3.8 cm, 22 gauge needles. Blood samples were placed on ice for at most 30 min until centrifuged at 1,800 rpm for 6-8 min. All plasma were collected and stored at -20°C for later analyses. When repeated blood samples were taken (as in experiment with estrogen implants), hematocrit values were determined prior to blood separation (Hesser 1960). The laboratory plasma analyses included measurements of estrogen, vitellogenin (alkali-labile phosphorus, ALPP), hematocrit, and total plasma calcium.

Determination of plasma vitellogenin:

Since purified vitellogenin of sturgeon antigen was not available at the initiation of this work, we utilized indirect techniques measuring concentrations of vitellogenin bound phosphate ions and total plasma calcium. Vitellogenin phosphate concentrations were determined as alkali-labile phosphorus (ALPP) using a modification of previously published techniques (Wallace 1970; Wallace and Jared 1968; de Vlaming et al. 1984). In white sturgeon, 92% of total plasma ALPP is incorporated into the vitellogenin fraction

with a molecular weight of 450 KD (Kevin Kroll pers. comm.). A 1 ml of trichloroacetic acid (TCA) was added to 200 µl of plasma. Plasma proteins were precipitated with TCA and centrifuged for 20 min. (9000 rpm, 4°C). The supernatant was poured off and pellet washed with diethyl-ether. In a dry heating block (102°C), the protein bound phosphorous was then liberated from pellet with 0.25 ml of 1N NaOH. The mixture was then neutralized with 0.25 ml 1N HCL, and the protein was again precipitated with 0.5 ml 24% TCA. After centrifugation the liberated inorganic phosphorous was determined in supernatant by the ammonium molybdate and chloride hydrazine reaction adapted to an autoanalyzer (Technicon Auto Analyzer; 660 nm in 15 nm flowell). Total plasma calcium was determined in 50 µl samples by atomic absorption spectrophotometry (Perkin- Elmer). Plasma samples were diluted in 0.1% Lanthanum and read at 422 µm wavelength.

Radioimmunoassay (RIA) for plasma estrogens:

Plasma estradiol (E2) was quantified by RIA modified from a procedure reported by de Vlaming et al. (1984), based the technique described by Tillson et al. (1970). The steroid was extracted from 50 µl of plasma using 20 volumes of diethyl-ether after immersion in a dry-ice acetone bath and vortexed for 2 min. The ether phase was decanted into a tube, evaporated to dryness and reconstituted in 100-500 µl of 0.01 M phosphate buffer (PBSG); pH 7.0. Mixing time between extraction and incubation was extended over 12

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hours. Recovery was 85-90%. Anti-estradiol (GDN-244-anti E2-6-bovine serum albumin supplied by Dr. G.D. Niswender, Colorado State Univ., Fort Collins, CO) was diluted 1:10,000 in 100 µl PBSG and incubated 30 min at room temperature. This dilution allowed 30-50% binding of labelled steroid in the absence of unlabelled steroid. The anti-E2 serum cross-reacts 15% with estrone as reported by Korenman et al. (1974). Sturgeon plasma was not chromatographed to separate E2 metabolites; therefore, the concentrations reported represent total estrogens. Labelled steroid ([2,4,6,7-3H] estradiol, 0.1 µCi, New England Nuclear) was added, and samples incubated overnight at 4°C. Separation of free from bound steroid was achieved using a suspension of 0.0625% Dextran and 6.25% charcoal PBSG. Standards were prepared from E2 (Sigma) and minimum assay sensitivity was determined as 0.25 ng/ml. Intra- and inter- assay coefficient of variation, using plasma pools collected from mature and immature sturgeon were 5.26% and 9.56%, respectively.

Gonadal histology:

Gonadal tissue samples were fixed in 10% phosphate buffered formalin. Diagnostic slides were prepared following standard histological procedures to those outlined by Thompson and Hunt (1966). The sections were placed in a vacuum infiltration processor (VIP 2000), dehydrated in alcohol, and embedded in paraffin following a schedule of 45 minutes in each bath. Blocks were sectioned at 5-8 µm, and stained with hematoxylin and eosin for histological

examination. Some selected sections were stained with Gomori's trichrome and the periodic acid-Schiff reaction (PAS).

Evaluation of gonadal development and gametogenesis:

Stage of sexual maturity was evaluated macroscopically and histologically. Macroscopic (or visual) evaluation were used to approximately determine stage of maturity in the field, and in general, provided reliable background information on sex and selection of fish for spawning. The criteria used for this evaluation are given in Table 1.

Table 1. Criteria for macroscopic evaluation of maturity stages of white sturgeon.

Maturity stage	Criteria: Ovaries and Testes

UNDIFFERENTIATED GONADS:
Thin and elongate gonad, with slender sex chords and small fat deposits. Usually in smaller than 76 cm wild caught fish, and younger than two years domestic stock.

OVARIES:

Stage I. Immature:
Moderate size organ with adipose tissue occupying more than half of gland. Ovigerous folds are granular in texture, and discernable in distal and lateral portion of organ; yellowish-pink in color.

Stage II. Recrudescing:
Area of the ovarian fold expands onto lateral surface of organ. Germinal tissue of yellow-pink color with small but visible (<1 mm) translucent oocytes.

Stage III. Maturing:
Enlarged and well vascularized ovary; reduced adipose tissue. Medium size oocytes (1.0-2.5 mm). Oocytes white in color, transforming into yellow-brown to light gray. *

Stage IV. Ripe:
Ovary fills peritoneal cavity with 3-4 mm, black tinted oocytes.

* In females which previously have spawned (or matured), dark pigmented granules can sometimes be observed in the ovary: the "salt & pepper" condition.

TESTES

Stage I. Immature:
Thick and elongate organ consisting principally of dense and fatty adipose tissue. Relative thin, white color germinal chords are located in dorsal part of organ.

Stage II. Developing:
Large gland, smooth in texture with dark pigmented tunica. White chords increase in volume.

Stage III. Maturing:
Turgid organ consisting of mainly white germinal tissue; dark but translucent tunica.

Stage IV. Ripe:
Distended and lobulate gland of creamy-white color.

Microscopic examination of histological sections provided material for study of gametogenesis and more precise evaluation of stage of maturity (maturity index). Slides were examined with a compound microscope, at magnifications ranging from 200-630x. A score was assigned to each section according to the stage of meiotic division within each testicular cyst and morphogenetic structures of the ovarian follicle. In testes, four general cell types were reliably recognized: spermatogonia, spermatocytes, spermatids, and spermatozoa. Based upon the stage of development in each cyst, the number of each cyst type per several fields of view were obtained. In females, ovarian stages were quantified by recognizing discrete morphological structures or characteristics of the follicle (e.g. layers of follicular and egg envelopes, yolk platelets, other cytoplasmic inclusions and cytoplasmic staining properties). The sum of individual parameters scores gave the final reproductive index for individual fish. A weighted score was also used where a multiplication factor was assigned to each examined parameter, increasing in developmental progression. Using unweighted or weighted individual scores, progress in reproductive development from the initiation of meiosis to spawning in individual fishes can be clearly seen and serve for quantification of gametogenesis (Figs. 1). Based on gross and more precise histological examination of the testes and ovaries, the population was then divided into immature, maturing, and ripe fish.

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The histological score ranges for the different stages of maturity were:

	Females (unweighted)	Males
Immature:	2-22	2-4
Maturing :	14-34	5-24
Ripe :	32-60	25-32

For overlapping scores, the absence (immature) or presence of yolk (mature) defined the stage. Between maturing (centered germinal vesicle) and ripe (polarized GV) oocytes the position of the germinal vesicle defined the stage.

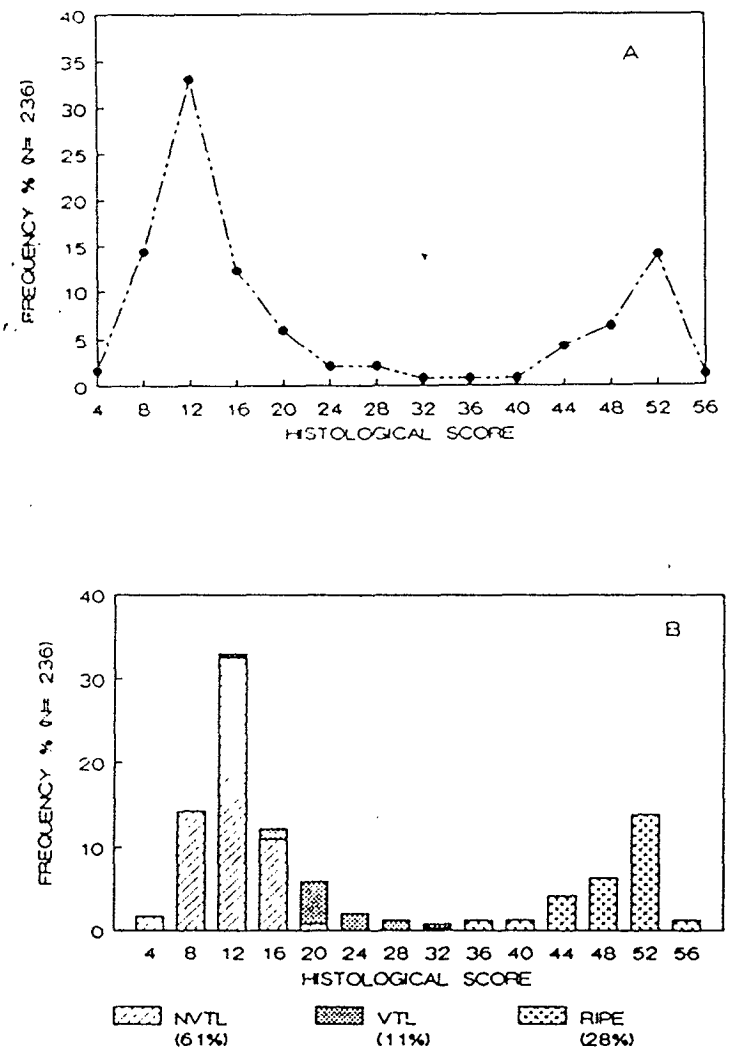

Figure 1. An example using the histological score. (A) overall distribution. (B) Emphasizing stages of oogonial development. NVTL-non-vitellogenic; VTL-vitellogenic; ripe gonad.

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Experimental Protocols

Semen evaluation: The objective of this experiment was to measure the fertilizing ability of semen of domestically raised sturgeon compared to that of wild caught males. A semen evaluation and percentage of eggs fertilized were the variables used to detect differences among males. The relationship between the various semen characteristics and the proportion of eggs fertilized was determined using a factorial experiment with three replications. A total of ten mature males, five of wild stock and five of domestic stock 2-3 years of age were used in this experiment. Three females of wild stock were induced to ovulate and the eggs were inseminated by each male used in trials. For semen collection, the males were repeatedly hormonally induced to spermiate for a period of five weeks.

Spermiation was accomplished with injections of carp pituitary extracts. Depending on water temperatures, ripe males responded well to dosages of 2.5-3.0 mg/kg administered in two applications (10% priming dose, 90% resolving dose, respectively). Spermiation was expected 12-16 hours after resolving dose. Semen was drawn with a large syringe (e.g. 20 cc) fitted with a short piece of tygon tubing attached to a tip cut from a disposable transfer pipette. Care was taken to prevent contamination of semen with water. Collected milt was then transferred to glass vials placed on crushed ice. Once collected, semen was stored dry at 4°C until used in the fertilization trials.

Sperm density, overall motility, and duration of motility were evaluated. Semen sub-samples were diluted 1:20, 1:40, 1:200 with saline, and cells were counted in a hemocytometer. The motility of the spermatozoa was score by placing a small drop of milt on a covered microscope slide and mixing it with a measured amount of fertilization water. Once the sperm was activated, overall motility and duration of motility were estimated. Three time intervals and making a series of yes-no decisions was the procedure used. At approximately 20 sec (time lapse for dilution and initial observation), are 8 of 10, or 5 of ten sperm motile? The second, reading (time), when 50% of the sperm were motile, and final value (duration of motility) when most all spermatozoa (>99%) ceased their motility. Tests were run in triplicate, and only progressive motility was considered. Fertilization was conducted in 150x15 mm petri dishes containing approximately 200 ova. Dishes were placed in 1.2 m circular tanks supplied with flow-through, well aerated 16 °C temperature water. Eggs in each dish were fertilized with 20 ml of a sperm suspension; a dilution 1:200 semen to water was used to avoid polyspermy. After approximately 6.6-7 hours, the adhering eggs were removed, fixed in 4% buffered formalin, and examined for presence of cleavage. Presence of the eight cell stage was used as evidence of normal fertilization (Detlaf et al. 1981.).

Experiment on the effect of estradiol-17β on vitellogenesis synthesis:

The aim of this experiment was to obtain background information for future studies on the influence of estradiol-17β on vitellogenin production. We used six immature fish (five males, one female) from the 1980-1983 year classes and ranging 6-10 kg in body weight. Groups (n=2/group) of sturgeon were sham operated or implanted with cholesterol-cellulose pellets (Innovative Research of America; Toledo, Ohio) containing 17β-estradiol and monitored for a period of 35 days. Individual pellets were implanted surgically into the peritoneal cavity. Blood samples (10 ml) were taken between 0700 and 0900 hours every 7 days. The pellets were prepared to contain approximately 2, 4, and 15 mg per body weight of 17β-estradiol and last 21 days at 15°C. During the course of this study, the animals were contained in tanks holding freshwater at temperatures between 9-13°C.

Statistical Analyses

Procedures described in Box et al. (1978) and Steel & Torrie (1986) were followed. The Statistical Analyses Systems Program (SAS Institute, Inc. 1986) was used for microcomputer processing. Analyses were performed on raw data or log transformations and unless specified were expressed in standard error of the mean. Comparisons between means, relationships and differences between regressions were tested using Student t test, analysis of variance (ANOVA) and linear regression analysis. Tests of

significance were made at the 0.01 probability level. The particular tests for each experiment are described appropriately in the results section.

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RESULTS

The results are presented in three sections. First section describes the gonadal anatomy and gametogenesis, based on observations of wild sturgeon from San Francisco Bay. Second section provides information on the reproductive state and gonadal cycles of wild sturgeon broodstock. The third section provides initial data on growth and sexual maturation of F1 captive sturgeon raised in University of California, Davis hatchery.

I. GONADAL STRUCTURE AND GAMETOGENESIS

Gonadal Anatomy

The paired gonads of adult male and female white sturgeon were attached to the dorsal peritoneum by mesenterial folds, and extended longitudinally from the pericardial region to the posterior wall of the pelvic cavity (Fig. 2). The gonadal ducts developed in close association with the kidneys. Both sexes retained the paramesonephric (or Mullerian) ducts, which served as an oviduct in females. The passage of sperm was provided by the connection of the efferent ducts of testes to the main kidney duct. Both paramesonephric ducts and kidney ducts opened in the urogenital sinus. The testis was an enclosed organ, covered by the tunica and organized as an acinar structure. The female glands were paraovarian and consisted of lateral and medial lobes, with the ovigerous epithelium

open into the coelomic cavity. During the gametogenesis, the ovaries and testes underwent dramatic changes in shape and size, schematically shown in Figure 3. Indifferent gonads were thin sex chords consisting of adipose tissue and germinal epithelium, with embedded germ cells. Prior to further differentiation, the sex chords enlarged due to proliferation of adipose tissue. Differentiation of the ovary was recognized by the appearance of a longitudinal groove on the lateral side which later expanded into the lateral and medial ovigerous folds. Undifferentiated germ cells were assembled in the ovigerous lamellae (or skeins) of females, and within a thin chord of the dorsal testicular portion in males. Testes do not exhibited external anatomical changes.

Cytological sex differentiation (differentiation of gametocytes) began soon after ovarian differentiation. The germinal tissue proliferated from the lateral groove of the ovary and dorsal portion of testes, concomitant with enlargement of the gonads, the absorption of adipose tissue, and gametogenesis (Fig. 3). In the maturing female the ovary was enlarged, vascularized, and the amount of adipose tissue greatly diminished. Growing follicles were initially white in color but acquired a dark pigmentation after reaching approximately 2 mm in diameter. In a ripe female the ovary filled the entire peritoneal cavity and the oocytes were grayish-black, 3.5-4.0 mm in diameter. At ovulation, follicles ruptured and the oocytes escaped into the ciliated

peritoneal cavity. During the oviposition, the eggs were shed via the paramesonephric duct. The latter possessed a muscular sphincter located distally from the infundibulum, which apparently controlled the passage of eggs. In males, the testes were smooth white organs apposed by a thick layer of adipose tissue. Immature testicular tissue retained the appearance of sex chords, covered by the darkly pigmented connective epithelium. When testes developed, the dark connective tissue stretched, and the adipose fatty tissue was replaced with more solid white testicular lobes. Thin fibrous septae divided the gland into many compartments (lobules) containing cysts with developing gametocytes. Mature testes were creamy white, solid, and lobed structures. During spermiation, ripe sperm passed through ductules (efferent ducts) entering the kidney duct. Adult white sturgeon were gonochoristic: among the total number of 836 fish sampled in San Francisco Bay for gonadal histology, we observed no cases of hermaphroditism. However, except for ripe fish on spawning grounds (females recognized by enlarged abdomen and male by the spermiation), adult white sturgeon do not exhibited external sexual dimorphism. We found no statistically significant difference between two sexes in distributions of dorsal and lateral scutes, relative length of head, snout, and width of mouth (Table 2). The only significantly different character was number of scutes in ventral row (greater in males, Table 2). Sturgeon male had greater number of scutes in ventral,

dorsal and lateral rows; however, there was a substantial overlap between the two sexes (Table 2 and Figure 4).

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Table 2. Morphologic characters of adult sturgeon sampled in San Francisco Bay.

	FL cm	Wb Kg	Sd(1) ##	Sl(1) ##	Sv(1) ##	Ll(2) %	Ls(3) %	WM(4) %
A. Females (n= 23)								
Range	84-164	5.4-43.5	8-13	32-45	8-12	17.8-28.5	29.6-43.5	25.9-42.4
Mean	131	22	10.52	38.13	9.7	20.8	34.6	35.8
S.E.M.	5	2.2	0.3	0.7	0.2	0.3	0.6	0.7
B. Males (n=.28)								
Range	79-166	6.7-49.4	9-13	32-47	9-13	18.7-22.2	29.3-41.4	30.4-41.
Mean	124	21	10.07	38.86	10.5	20.2	35.6	36.1
S.E.M.	4.6	2.2	0.2	0.7	0.	0.2	0.5	0.5

1) Number of scutes in dorsal, lateral and ventral rows.
2) Length of head, percent of FL.
3) Length of snout, percent of Ll.
4) Length of mouth, percent of Ll.

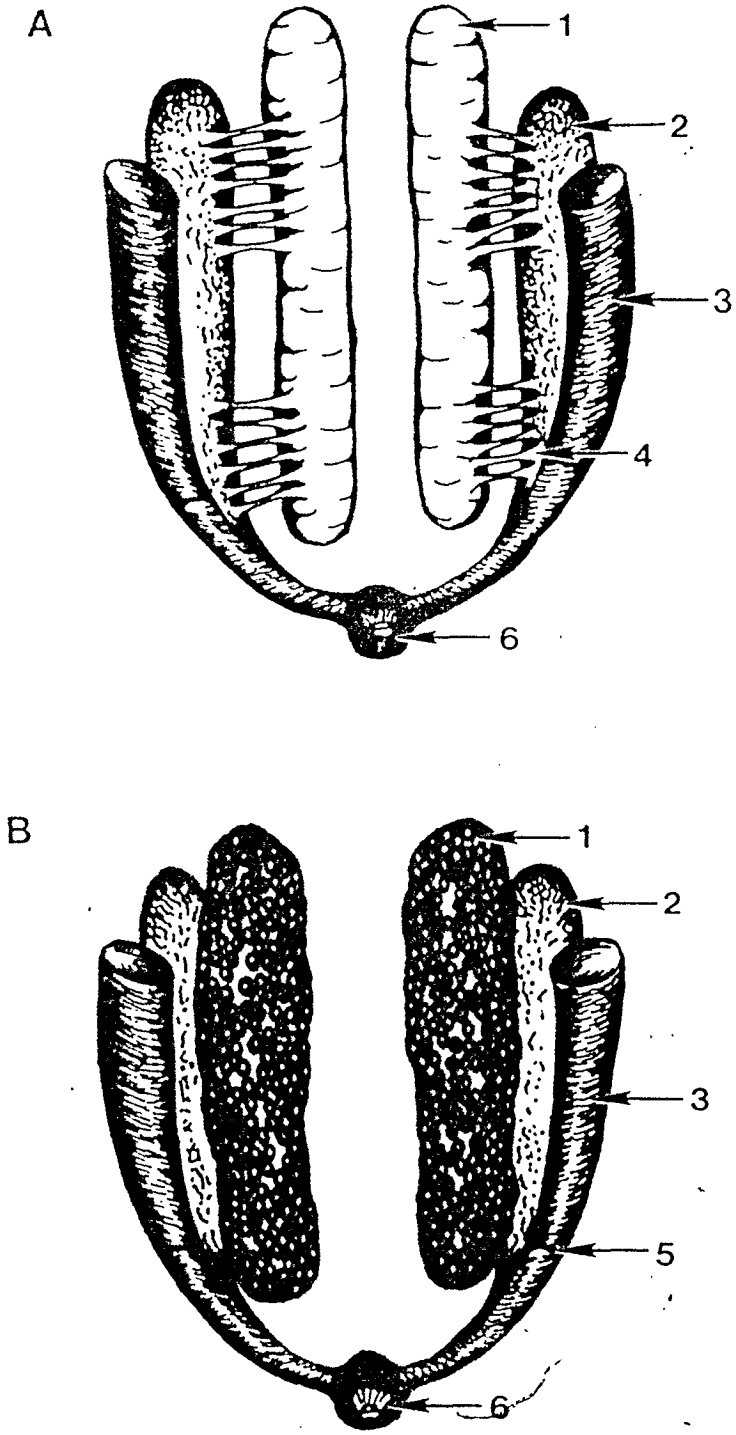

Figure 2. A schematic presentation of the reproductive system in white sturgeon male (A) and female (B). 1) gonads (testes or ovaries); 2) kidneys; 3) paramesonephric ducts; 4) efferent ducts; 5) muscle sphincter; 6) urogenital sinus.

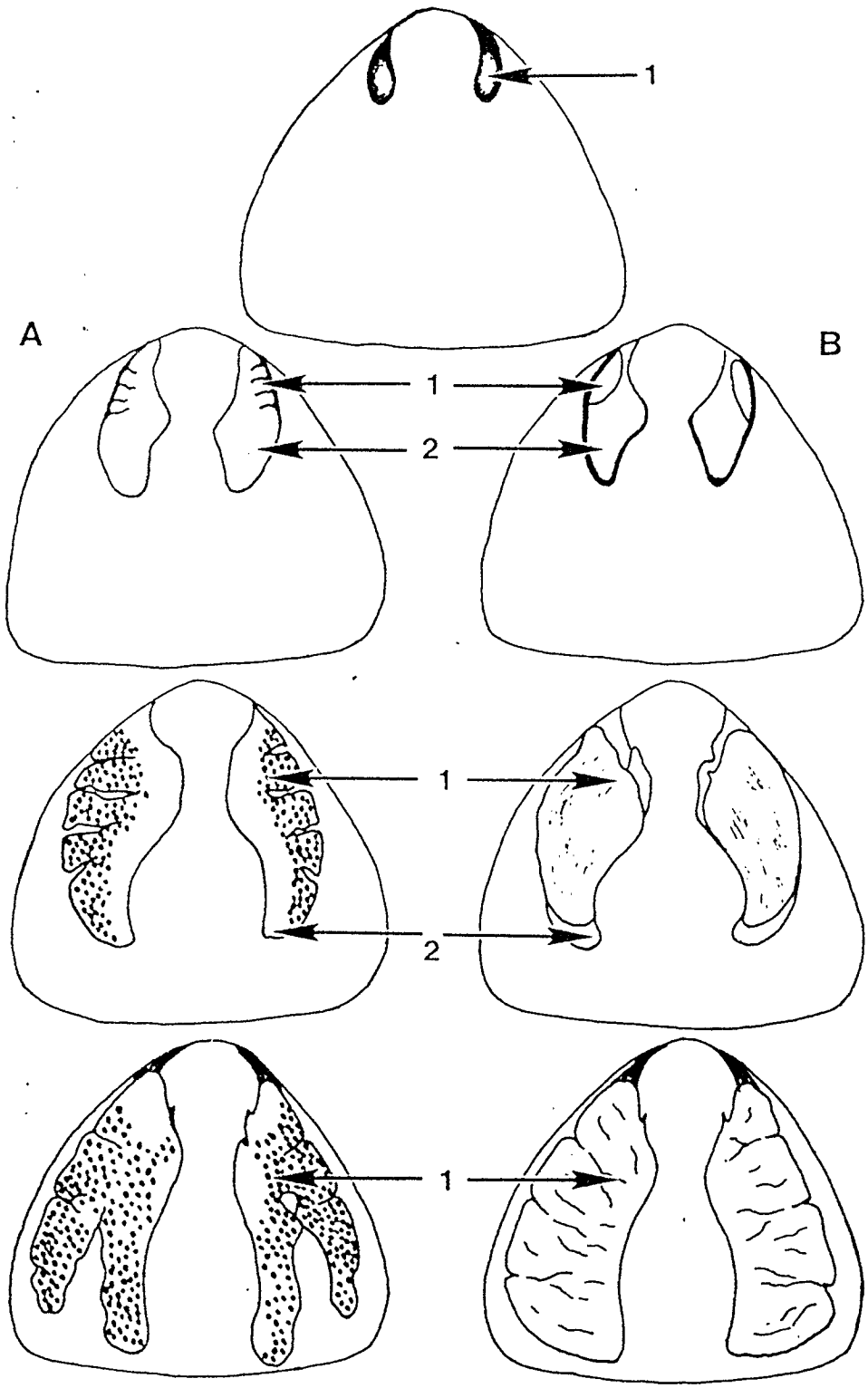

Figure 3. Scheme showing changes in shape and size of sturgeon gonads during gametogenesis. (A) female. (B) male. 1) germinal tissue; 2) adipose tissue.

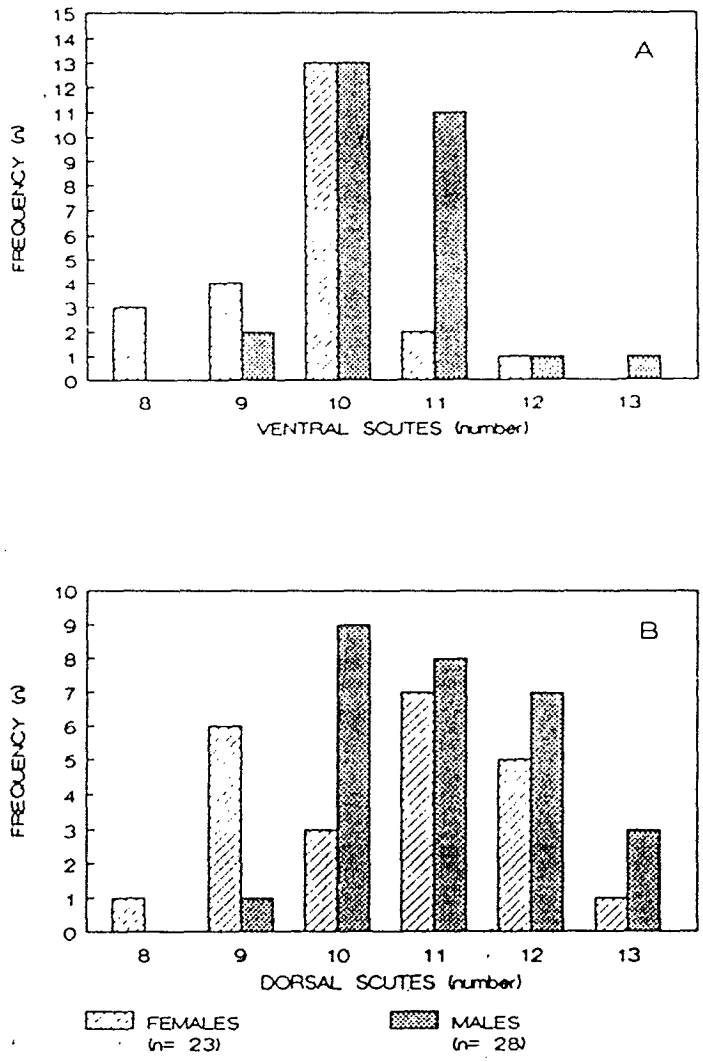

Figure 4. Distribution of the number of scutes in ventral (A) and dorsal (B) rows in adult male and female white sturgeon.

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Oogenesis

Various classifications have been used to describe the ovarian stages in sturgeon (e.g. Nedoshivin 1924, Cuérrier 1966, Lukin 1941, Magnin 1966, all reviewed by Roussow 1957 and Goyette et al. 1988). Stages of the ovarian development established by these classifications, on the external appearance of the ovary, egg size, pigmentation, and distribution of adipose and germinal tissues. Such characters may not accurately reflect gametogenesis. Based on light microscopy examinations of the ovarian sections, we divided the oogenesis observed in wild stock of San Francisco Bay into five stages:

Stage 1: Gonial proliferation and differentiation of the primordial follicle.

Stage 2: Cytoplasmic growth of primary oocyte and resting stage of primordial follicle.

Stage 3: Pre-vitellogenic growth of primary oocyte and differentiation of follicular envelope and zona radiata.

Stage 4: Exogenous vitellogenesis and major growth of primary oocyte.

Stage 5: Polarization of eggs and maturational events.

The description of these stages follow.

Stage 1. Primary Oocytes:

This stage encompassed period of gonial proliferation and differentiation of the primordial follicle (Fig. 5). Stage 1 was found only, in 2-4 years of age domestic females following gonadal sex differentiation. A cross-section of the ovary reveals a thin surficial layer of gonial and fibroblast cells (germinal epithelium), laterally apposing a thick layer of adipose tissue; the germinal tissue was separated from the peritoneal cavity by a single layer of epithelial cells. Gonial cells were aggregated in groups. Embedded deep in the germinal tissue were the larger (25 μm) primary oocytes. Usually located adjacent to adipocytes, primary oocytes had a large, dark nucleus, and a narrow, homogeneous, light staining (with hematoxylin) cytoplasm. Often, the nucleus contained condensed chromatin material, indicating oocytes had initiated the meiotic prophase (synaptic phase). Each oocyte was surrounded by a thin membrane, and several fibroblast cells.

Stage 2. Non-proliferating or resting, primordial follicles:

The follicles at this stage of development were observed in all adult females, including post-spawning animals. In the females entering pre-vitellogenic, and vitellogenic conditions (stages 3 and 4), only a portion of the oocytes entered the growth and development, while the rest of ovarian follicles remained in stage 2. Hence, our definition of "resting" stage. During this stage of development the germinal portion of the ovary grew, and

formed clearly visible ovigerous lamellae. Resting primordial oocytes became visible to the naked eye as they attained 200-400 μm in diameter. The nucleus (150 μm) was light staining and the nucleolus dispersed into many nucleoli. The cytoplasm of the oocyte was dense and homogeneous, and acquired strong basophilic properties, indicating RNA and protein synthesis. Resting follicles were recognized by numerous, large and spherical vacuoles residing in the cortical area of the cytoplasm (Fig. 5). These conspicuous vesicles did not stained with H & E or the PAS reaction, and their chemical nature remains unknown. The oocyte was surrounded by rudimentary follicular granulosa and thecal cells, and a discrete glycoproteid basement membrane, the basal lamina. Primordial granulosa cells appeared as several undifferentiated fibroblast-like cells, situated between the oolemma and basal lamina. The basal lamina was an extracellular supporting matrix, that exhibited strong PAS positive staining that was not affected by amylase digestion, and therefore, consisted of glycoprotein. Outside of the basal lamina were flat, plate-like, primordial thecal cells. The intersticial space between the follicles was filled with adipocytes, blood vessels, and melanocytes.

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Stage 3. Folliculogenesis or pre-vitellogenic oocytes:

Observations in wild caught females and one domestic female, indicated that pre-vitellogenesis was initiated in the fall (October-December), and occurred within a short period of time (few months or less). This period was followed by the initiation of exogenous yolk deposition (i.e. vitellogenesis). The transition from a resting stage to pre-vitellogenesis involved cytological changes in the oocyte, the follicular cells, and the adjacent connective tissue. However, the major events which marked the beginning of this stage were differentiation of the granulosa cell layer and initiation of secretion of the zona radiata (i.e. folliculogenesis). As a result, two types of oocytes were distinguished in the ovary. One type comprised the resting oocytes which remained in stage 2 during vitellogenesis. A second type was a group of eggs which separated from a resting pool of primordial follicles and entered a pre-vitellogenic growth and differentiation (Fig 6). During pre-vitellogenesis, the ovarian fold expanded onto the lateral surface of the ovary and oocyte diameter increased to 300-500 μm. This was accompanied with a reduction in adipose tissue. The lamellae projecting into the coelomic cavity were covered by a vascularized peritoneal epithelium. The cell nucleus (250 μm) contain dispersed chromatin material and a large number of densely stained nucleoli in the periphery of the nucleoplasm. In the cytoplasm, the peripheral vacuoles gradually disappear, and a network of

multi-vesicular structures imparting a "rete" web-like appearance are formed around the nucleus. The ooplasm remains basophilic but exhibits much less dense staining compared to the resting oocyte. In pre-vitellogenic oocytes, the follicular envelope consisted of granulosa and thecal layers separated by the basal lamina, and an amorphous zona radiata interposed between the oolemma and granulosa cells. Cells of granulosa layer proliferated and differentiated into a simple cuboidal epithelium. The theca layer contained occasional blood capillaries. The zona radiata stained positively with the PAS reaction, and appeared to consist of an outer and inner layer, both of glycoprotein nature (Fig. 7).

Stage 4. Vitellogenesis:

Exogenous vitellogenesis is manifested by yolk deposition (Fig. 8). This process involves secretion by the liver of yolk precursor vitellogenin, and its subsequent uptake by the oocytes. During vitellogenesis pigmented granules were also deposited in the cortical cytoplasm of the oocyte and gave the characteristic grayish-black color of ripe sturgeon eggs (Fig. 9). Although exact duration of vitellogenesis in sturgeon is unknown, our observations on wild stock indicated this stage has a duration of approximately one year, and was completed 3-6 months before spawning. Exogenous vitellogenesis may be subdivided into three substages:

Early vitellogenesis- Early vitellogenic eggs were about 0.5 mm in diameter and acquired a characteristic white color. The gonad was also highly vascularized. The cytoplasm showed a transition from basophilic to eosinophilic staining, and possessed numerous inclusions. Yolk first stained with the PAS reaction, and appeared in the periphery of the ooplasm in the form of small irregularly shaped bodies. As yolk bodies scattered throughout the cytoplasm, they crystallized, and formed yolk platelets. Closely associated with yolk were small oil droplets. The nucleus of these oocytes was small and centered and contained randomly distributed nucleoli. The nucleus was also surrounded by a dense perinuclear zone or 'cloud' (Fig. 6 & 8). Studies in teleost fish suggest that this 'cloud' may be numerous clusters of mitochondria and stacks of smooth endoplasmic reticula. Oocytes in this stage resided within a distinct follicle. Follicular cells proliferated and were well differentiated. Granulosa cells were cuboidal, and numerous blood capillaries penetrated the thecal layer. Thin striations developed in the zona radiata reflecting interdigitations of granulosa cell cytoplasmic processes and the microvilli of the oocyte.

Mid-vitellogenesis- Figure 9 depicts a typical vitellogenic oocyte. This phase was distinguished by the rapid growth of the oocytes, probably an indication of increased vitellogenin uptake by the oocytes. The cytoplasm possessed numerous inclusions and was entirely acidophilic in nature.

While gradually expanding towards the nucleus, both yolk platelets and oil spheres continued to accumulate and increase in size. The nucleus enlarged to above 0.3 mm in diameter. The nucleoli were less prominent and dispersed from the periphery into the interior of the nucleoplasm, Numerous beaded strings of chromatin material were visible soon thereafter. The zona radiata thickened and differentiated into two layers, z.r. interna and z.r. externa.

Late vitellogenesis- The distinguishing characteristics of late vitellogenic oocytes were, pigmentation, differentiation of third layer of chorion and micropyles. Dark coloration of vitellogenic eggs began when the oocytes attained 2.5-2.7 mm in diameter and deposition of melanin pigments were observed in the cortical ooplasm (Fig. 9). The cytoplasm was filled with growing yolk platelets, and oil droplets. Aligned beneath the oolemma were numerous cortical alveoli. The follicular envelope thickened, and a third, "gelatinous" (terminology of Detlaf & Ginzburg 1955) layer had been secreted above the zona radiata. This layer stained intensely for carbohydrates with Masson trichrome. During the late stage of vitellogenesis the appearance of micropyles in the follicular envelope became apparent. Near completion of vitellogenesis, sturgeon oocytes were around 3.5 mm in diameter, spherical in shape, and uniformly black in color. The ovarian folds greatly extended, and filled up most of the abdominal cavity. The gonadosomatic index

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reached 10-20%, depending on individual fecundity and size of fish. In late fall, vitellogenesis appeared near completion when female broodstock were still intensively feeding in San Francisco Bay .

Stage 5. Oocyte maturation:

This stage encompassed polarization and final maturation of the oocytes. The following events occurred: re-arrangements in distribution of yolk and cytoplasm, changes in egg shape and color, and migration and breakdown of the germinal vesicle that occurred just prior to ovulation and oviposition (Fig. 10). Pre-maturational events, and polarization of sturgeon eggs extended during the period 3-6 months, of e.g during the spawning migration; spawning occurred in spring during March-April. At the initiation of oocyte maturation the cytoplasm of the oocytes was filled with yolk platelets of various sizes, oil droplets, and cortical alveoli. The nucleus or germinal vesicle (GV), was enlarged and occupied a centrally located position; no nucleoli were visible in the nucleoplasm. The oocytes were enclosed by three distinct investment coats containing several micropyles at the animal pole. Melanin pigment continued to be deposited in the cortex area of the oocyte. During the process of oocyte maturation, the shape of the oocytes changed from sphere to an oval (pointed), and the diameter of the oocytes changed from 3.4, to a final 4.0 mm, following the elongation of the animal vegetal axis. Large yolk platelets or "coarse" yolk (macro-platelets,

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≈20μ) and oil droplets were aggregated in the vegetal hemisphere; small yolk platelets or "fine" yolk (micro-platelets, ≈1-2μ) in the animal hemisphere. The germinal vesicle migrated from the center of the egg towards the animal pole, acquiring a pointed ellipsoid configuration. This migration progressed until the GV became fully submersed into the "fine" yolk area and reached the oolemma at the animal pole just beneath the micropyles area (Fig. 10). There were also substantial changes in color: the vegetal hemisphere was grayish-yellow and the animal hemisphere contained one or two concentric rings of dark pigment (Fig. 10).

The process of oocyte maturation was described in full details by Detlaf et al. (1981) and Detlaf and Skoblina (1969) for Russian sturgeons, and by Lutes et al. (1987) for the white sturgeon. The final maturational events involved the breakdown of the germinal vesicle membrane (GVBD), and mixing of nucleoplasm and chromatin with the cytoplasm of animal hemisphere. There was the reduction of the egg genome to 2n chromosome number associated with the resumption of the first meiotic division and concluded with separation of the first polar body. Ovulation occurred when the eggs were extruded from the follicular envelope into the abdominal cavity. This process involved the separation of the chorion from the granulosa layer and expulsion of the egg from the follicle aided by contractions of the theca layer. Final oocyte maturation and ovulation in sturgeon can be induced

in vivo by the administration of gonadotropin and gonadotropin releasing hormones. Maturation can also be induced in vitro, by short term incubation of sturgeon eggs in progesterone solutions; however, the ovulation was rarely observed in vitro. The mechanism of oviposition in sturgeons is poorly known. It involved complex movement of eggs toward the funnel of the oviduct by, perhaps, the ciliated peritoneal epithelium, and contractions of the abdominal musculature. Release of eggs into the environment probably occurs as a coordinated action of muscular contractions, abdominal pressure, and opening and closing of the sphincter in the ovarian duct. In sturgeon, the process of maturation and ovulation took several hours and progressed from the posterior to anterior portions of the ovaries (observations in vivo, by hormonally induced ovulations, and in vitro incubations). As eggs were ovulated, the female periodically discharges them on the bottom of the spawning tank.

Post-spawned ovary and atretic processes:

In white sturgeon, atresia or degeneration and absorption of oocytes occurred during all phases of ovarian development. However, atretic follicles were most common during pre- and early vitellogenic stage and after the ovulation (non- ovulatory or anovular eggs) (Fig. 11). During the oocyte resorption there was discoloration of the cytoplasm, and the nucleus took an irregular shape as it disintegrated. The stratified layer of the surrounding follicle collapsed and folded into the oocyte. Phagocytic

and absorptive activity were observed as granulosa cells hypertrophied and invaded the degenerating oocyte. If yolk was present, platelets lost their structural integrity and were absorbed. Pre-ovulatory atretic follicles were resorbed rapidly within a mass of hypertrophied granulosa cells, leukocytes, large fibroblasts, and strands of highly vascularized connective tissue. In old anovular eggs the yolk and cytoplasm had been absorbed, and the eggs were filled with discolored yellowish- brown to black pigmented granules. Granules remained amid connective tissue elements, and surrounded by the stroma of the ovary. The remnants of granules remained for prolonged periods of time but no epithelium or blood vessels were present around them. In early vitellogenic females (i.e. with white color oocytes), non-ovulated eggs were identified as black granules, and imparted a mottled appearance to the ovary, hence, the "salt and pepper" condition.

Spermatogenesis

There are some works, including the Russian sturgeon, A. guldenstadti (Ginzburg 1968) and white sturgeon (Cherr and Clark 1985), on the structure and function of the mature sturgeon spermatozoan. However, the testicular structure, and process of spermatogenesis in sturgeon are poorly described. While we focused major attention on female development, we provide below a brief classification of testicular stages, based on histological sections of most

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representative stages of spermatogenesis. This classification is merely for the convenience of determining the distinct stages of maturity by histological score, and does not give a complete picture of spermatogenesis. Three distinct stages were observed in wild fish captured in San Francisco Bay: Stage 1: Spermatogonial divisions and formation of spermatogenic cysts. Stage 2: Spermatogenesis (meiotic divisions of germ cells within the cysts). Stage 3: Ripe spermatozoa.

Stage 1. Immature or resting testis:

At the beginning of a new period of sexual cycle the gonial cells proliferate and produce generations of spermatogenic cells. Spermatogenic cells were grouped together in small cysts and were all at the same stage of development (Fig. 12A). Stem cells were large bodies characterized by irregular shaped nuclei, sometimes with chromatin granules, and pale staining homogeneous cytoplasm. Follicle cells (sertoli?) with flattened, irregularly shaped nuclei and pale staining cytoplasm were often seen enveloping the cysts.

Stage 2. Spermatogenesis:

In stage 2 the testis were undergoing active spermatogenesis (Fig. 12A). At some point, cells entered meiosis and spermatocytes were formed. Spermatocytes were smaller and stained more darkly. Numerous heterochromatic patches appeared in the nuclei. As development progressed the cells became much smaller and stained more uniformly.

Spermatids were recognized when cells became oval in shape. As elongation proceeded, spermatids underwent spermiogenesis and became mature sperm: elongated flagellated cell.

Stage 3. Ripe testis:

In stage 3, matured testes were turgid glands filled with cysts of spermatozoa (Fig. 12B). Cysts were surrounded by boundary cells and numerous blood capillaries. Spermiation involved bursting of cysts and secretion of seminal fluid. Semen was then discharged through efferent ducts leading into the kidney duct. Sperm appeared to be stored only in cysts, but on occasions were found in the lumen of ducts.

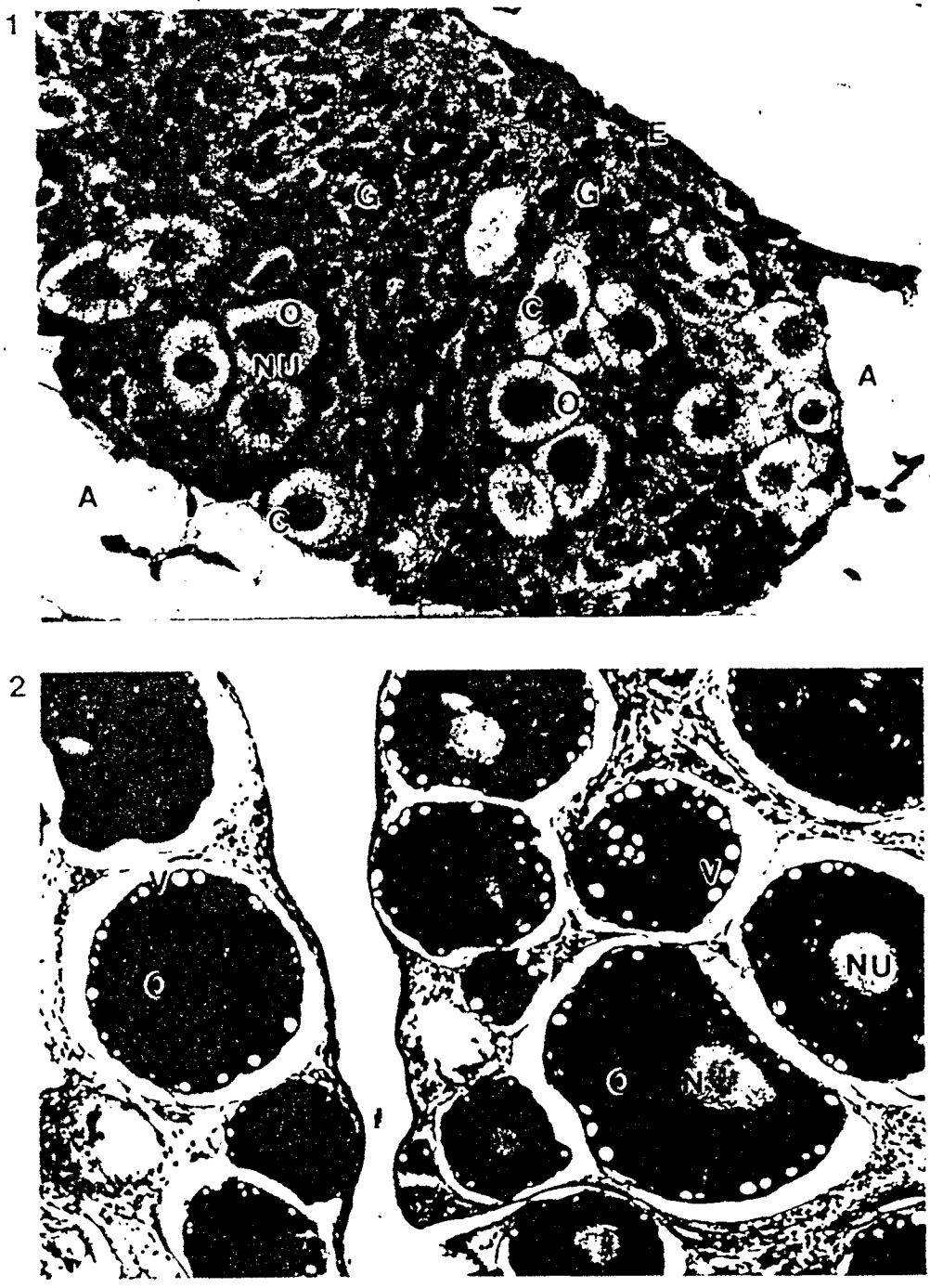

Figure 5. Stages 1 and 2 of oogenesis. (1) Primary oocytes. Adipose tissue (A); epithelium (E); gonial cells (G); oocyte (O); nucleus (NU) and chromatin (C). (2) Primordial follicle (O). Nucleoli (N); vacuoles (V). H & E. X400 and 250 respectively.

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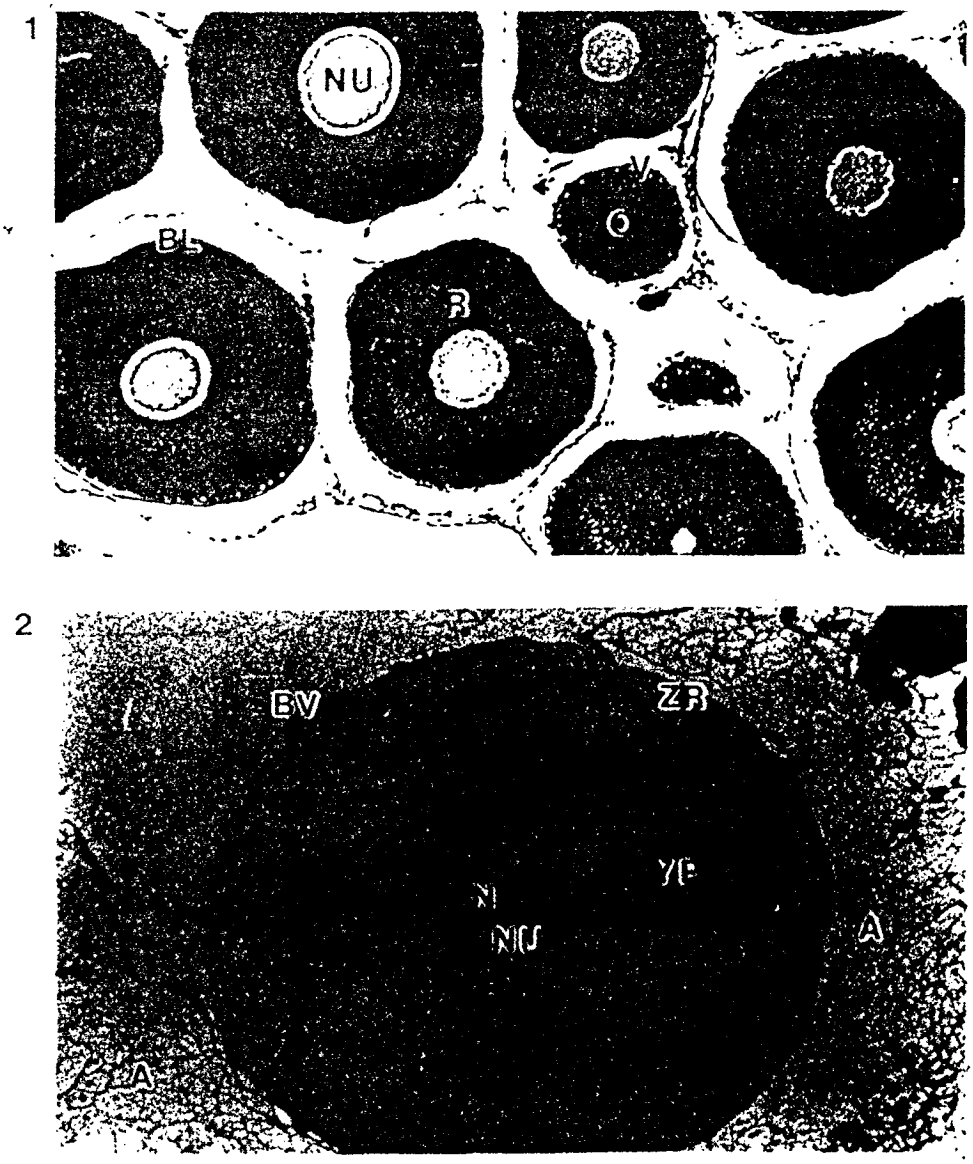

Figure 6. Stages 3 and 4 (early vitellogenesis). (1) Previtellogenic oocytes. Cytoplasmic reticulum (R); nucleus (NU); basal lamina (BL); primary oocyte (O) with vacuoles (V). (2) Early vitellogenic oocyte. Adipose tissue (A); capillary (BV); zona radiata (ZR) and yolk platelets (YP). H & E. X150.

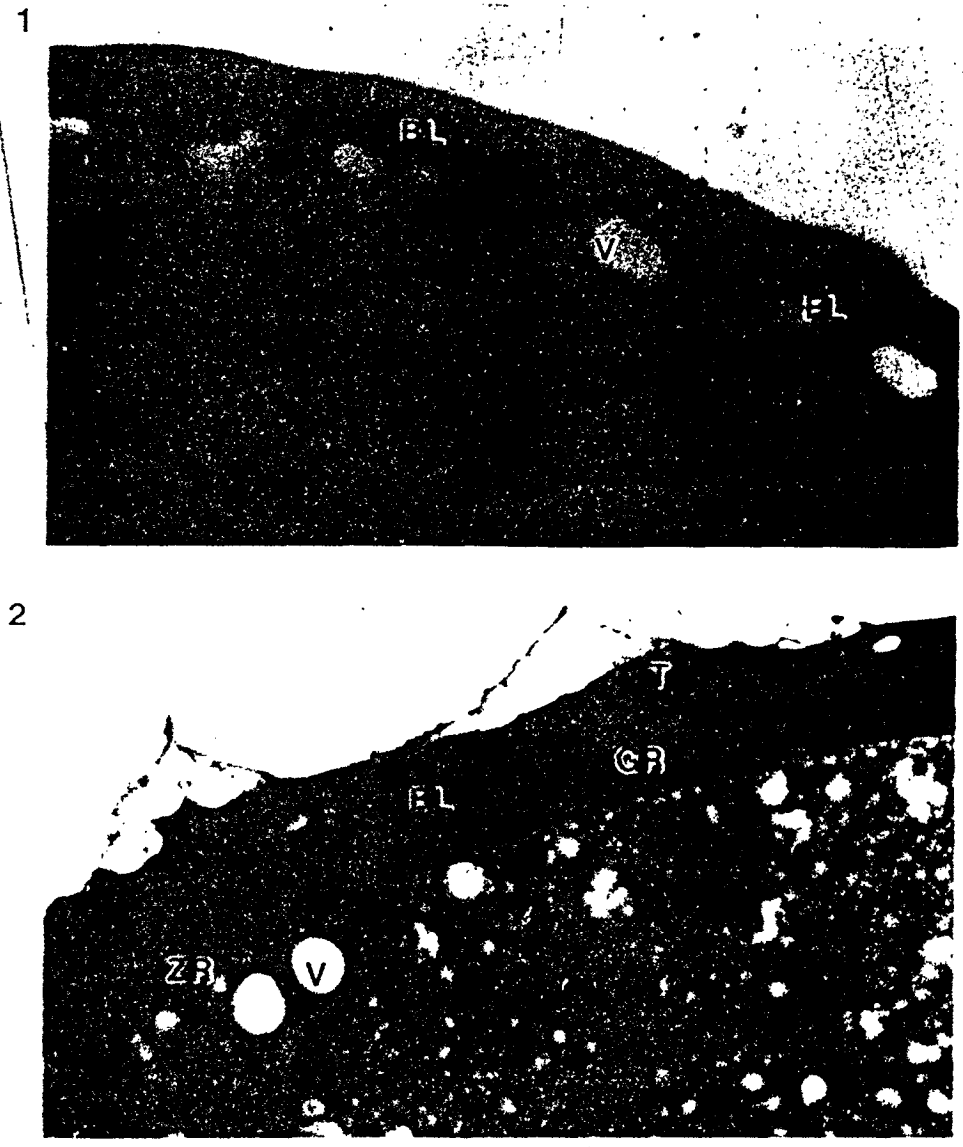

Figure 7. Structural changes in the follicular envelope observed during pre-vitellogenesis, prior to yolk accumulation; stage 3 to 4. (1) Peripheral section of rudimentary follicular envelope of primordial oocyte. Basal lamina (BL); fibroblast-like cells (FL); cytoplasmic vesicles (V). (2) Pre-vitellogenic oocyte differentiating follicular envelope. Basal lamina (BL); zona radiata (ZR); granulosa (GR) and theca (T) cells; vesicles (V). H & E, PAS, 1 and 2 respectively. X630.

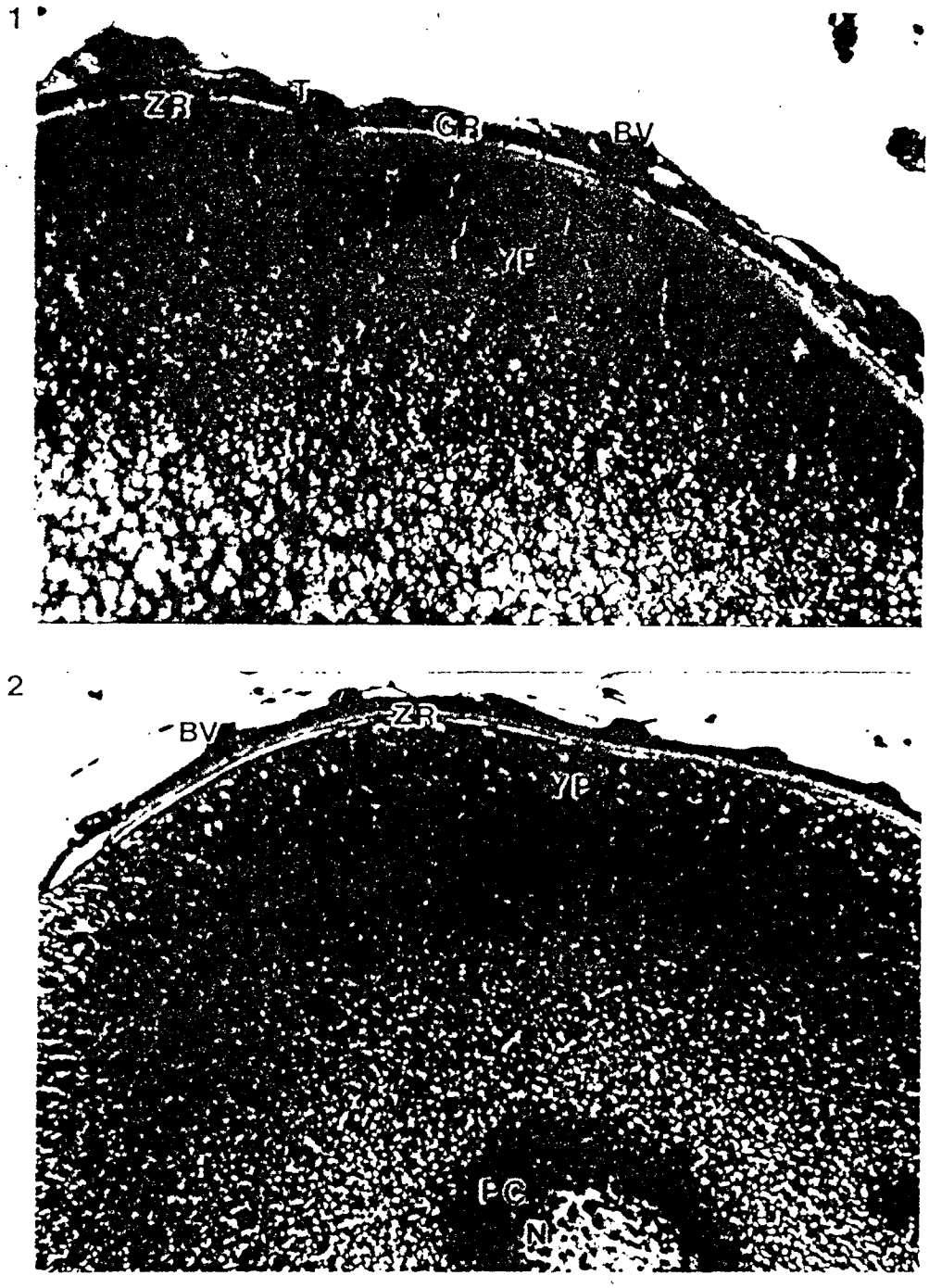

Figure 8. Early vitellogenic oocyte. (1) Note well differentiated granulosa (GR) and theca cells (T). Capillary with red blood cells (BV); yolk platelets (YP); just formed zona radiata (ZR). (2) Note dense perinuclear cloud (PC) and dispersed nucleoli (N). Cytoplasm is eosinophilic and beginning to fill with yolk. H & E. X250 and X150 respectively.

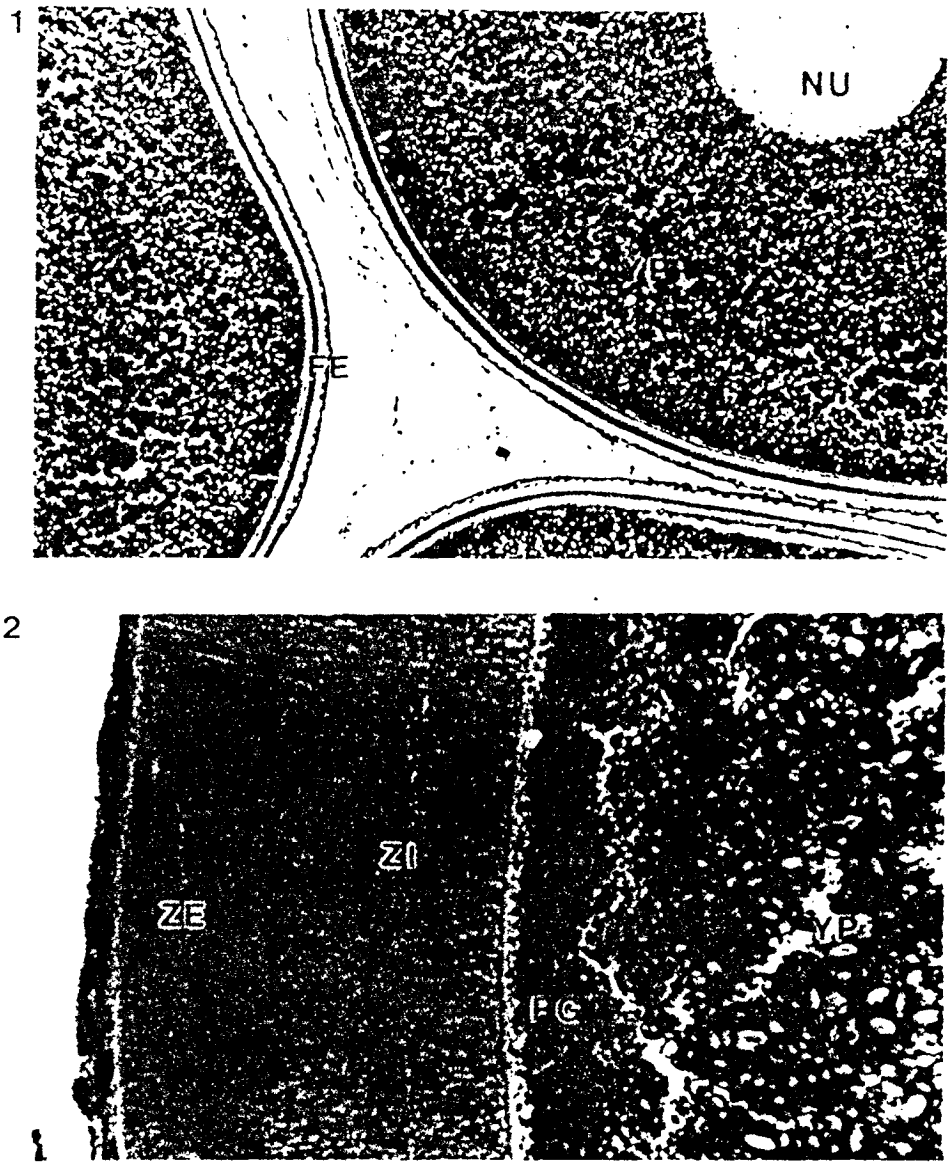

Figure 9. Vitellogenic oocytes. (1) Early vitellogenic follicle. Note nucleus with beaded thread-like chromatin. The cytoplasm is filled with yolk platelets and oil globules. (2) Late vitellogenic follicle. Note pigmentation. Follicular envelope (FE); nucleus (NU); yolk platelets (YP); zona radiata, externa (ZE) and interna (ZI); pigment granules (PG). H & E. X150 and X400 respectively.

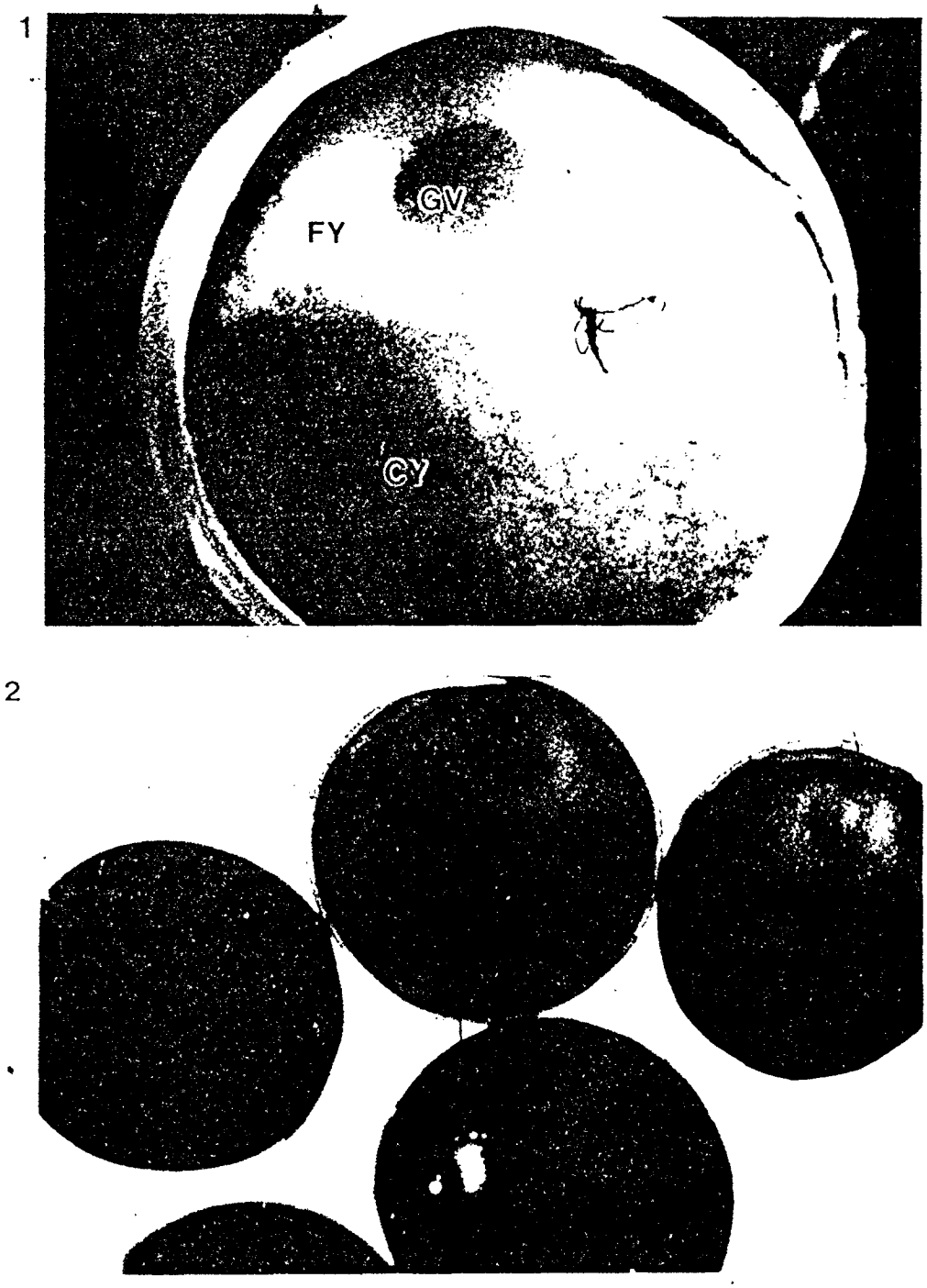

Figure 10. Stage 5 or oocyte maturation. (1) Cross section of mature oocyte. Note ooplasmic segregation and polarized germinal vesicle (GV); fine (FY) and coarse (CY) yolk platelets. (2) Oocyte maturation sequence. Note germinal vesicle breakdown. Prepared by heat fixation. X62.5 & X25 respectively.

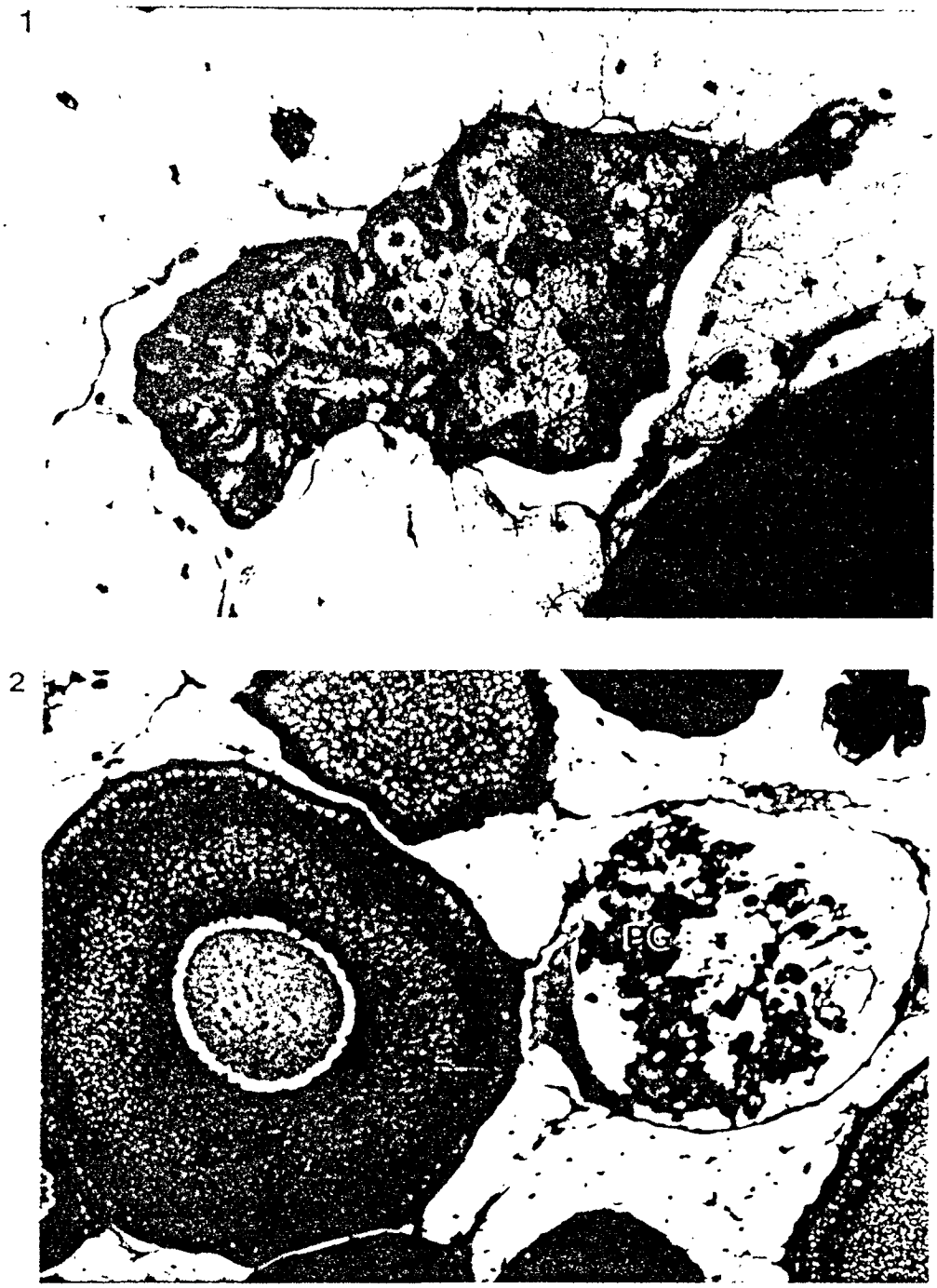

Figure 11. (1) Atretic and (2) degenerative follicles in sturgeon ovary. Pigment granules (PG). H & E. X250.

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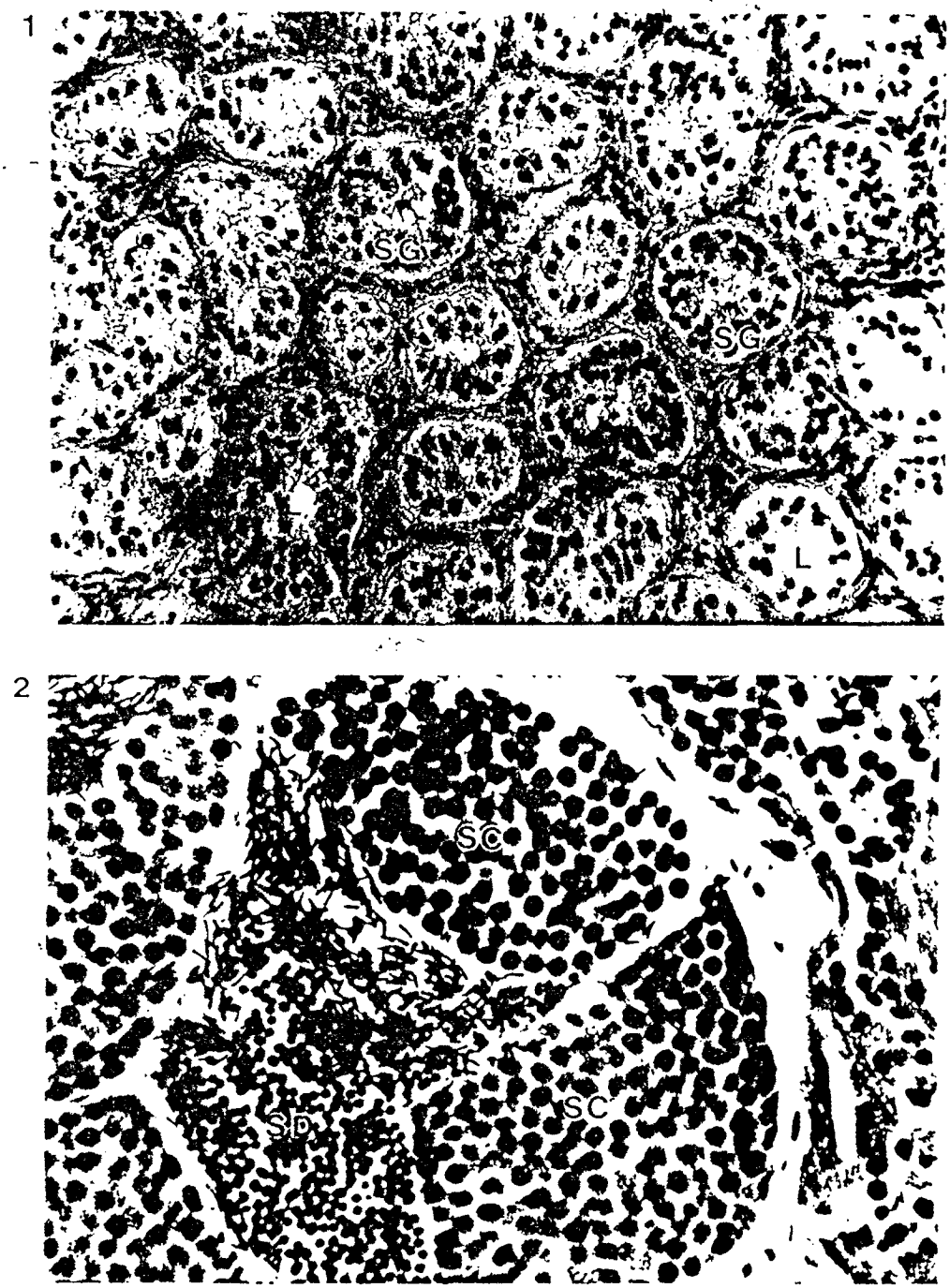

Figure 12A. Cross section through testis tubules.
1) Stage 1, spermatogenic cells (SG) are seen surrounding the lumen (L) of tubule. 2) Stage 2, progression from early spermatocytes (SC) to spermatids (SD) and through spermiogenesis. H & E. X630.

Figure 12B. Cross section through matured testis tubules; stage 3. Cysts contain just matured spermatozoa. H & E. X400.

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V. [illegible] OF STURGEON EGGS IN THE SAN FRANCISCO BAY ESTUARY

Size Distribution and Sex Ratio

In the San Francisco Bay Estuary, we captured a total of 836 sturgeon using broodstock collection methods. Fish ranged in size from 79 cm to 202 cm fork length. Mean length was 138.9 cm FL ±1.15 s.e.m. (Table 3). The length frequency data of the population had two modal groups which corresponded to size distribution of males and females (Figure 13). Mean fork length of females was significantly greater (145 ±1.15 cm, x ± s.e.m.), compared to that of males. Females above 155 cm FL were more numerous than males (133 cm FL ±1.03 s.e.m.). Males outnumbered females in size classes between 95 and 115 cm FL ($p < 0.05$). Between 115-155 cm FL the sex ratios were close to equality ($p > 0.05$). The overall sex ratio of the population was close to 1:1 (Table 3).

Size at First Maturity

The size or age at which white sturgeon reached sexual maturity was unclear. Neither sex showed gonadal development below 90 cm FL, and there were no ripe fish less than 100 cm FL. The smallest sexually matured male and female we found respectively were 12 to 14 years of age, and 110 cm and 104 cm FL. In San Francisco Bay, females tended to mature at a larger size (140-150 cm FL), and at a later age than males (120-130 cm FL) (Fig. 14).

Table 3. Frequency of male and female white sturgeon from San Francisco Bay within size classes FL, with Chi-square values assuming a 1:1 sex ratio.

Length (cm)	Males	Females	Chi-Squares
55-65	0	0	
65-75	1	0	
75-85	2	1	
85-95	6	4	
95-105	53	31	5.77
105-115	79	40	12.79
115-125	59	61	0.04
125-135	57	52	0.24
135-145	58	77	2.68
145-155	46	45	0.02
155-165	25	54	10.65
165-175	10	44	21.43
175-185	6	12	3.08
185-195	0	12	12.08
195-205	0	1	
P< 0.05	(Sex ratio 0.97:1)		
Total	435	421	

The Gonadal Cycle

The distribution of different stages of gonadal development in winter stock white sturgeon showed that most (44%) of the sturgeon caught were immature or in resting state of gametogenesis (Fig. 15 and 16). Gonads in active state of vitellogenesis or spermatogenesis were found in 31% of the animals. Only 28% of the population was mature and considered ready to spawn. The female population was composed of 74% non-vitellogenic and 11% vitellogenic individuals. Ripe females, considered ready to spawn, constituted a 15% of the female population, and only 8% of the total fish stock. Winter stock males predominantly (55%) maintained a constant stage of development (i.e. undergoing

spermatogenesis) or were ripe (37%). Only 9% of the male population had testis in resting or immature state.

The gonadosomatic index in white sturgeon coincided well with gonadal development (Fig. 17). High GSI values corresponded to ripe individuals, while low values reflected a period of gonadal arrest or early development. Mean GSI and maximum gonad weights were greater for females (26%) than males (5%). In males, overall GSI values were low and variable, which probably reflected the high amounts of adipose tissue deposited around the testes.

Changes in plasma levels of estrogen and vitellogenin in relation to the ovarian reproductive cycle of female white sturgeon:

In female white sturgeon, plasma estrogen and vitellogenin levels were extremely low in sexually immature and adult pre- vitellogenic fish; total estrogens were below detectable by RIA levels (0.25 ng/ml) and ALPP's were 4.76 μg/ml. Concentrations of both estrogen and ALPP significantly rose up to 4.76 ±1.3 ng/ml and 29.2 ±6.7 μg/ml respectively, in animals entering the vitellogenic stage, and remained elevated throughout vitellogenesis. At ovulation, concentrations of estrogens and ALPP fell to 0.29 ng/ml and 20 ng/ml respectively (Fig. 18). There was no correlation, however, between the estrogen and ALPP in group of individual fish undergoing ovarian recrudescence (Fig. 19).

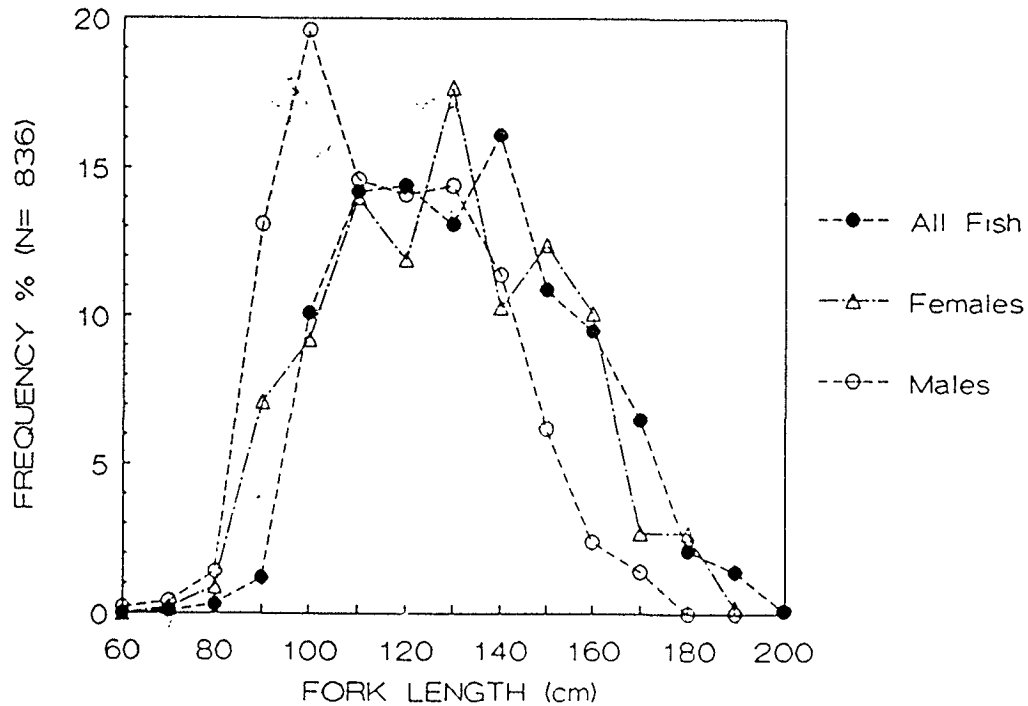


Figure 13. Size distribution in fork length (cm) of white sturgeon captured in San Francisco Bay during winter seasons 1979-1985.

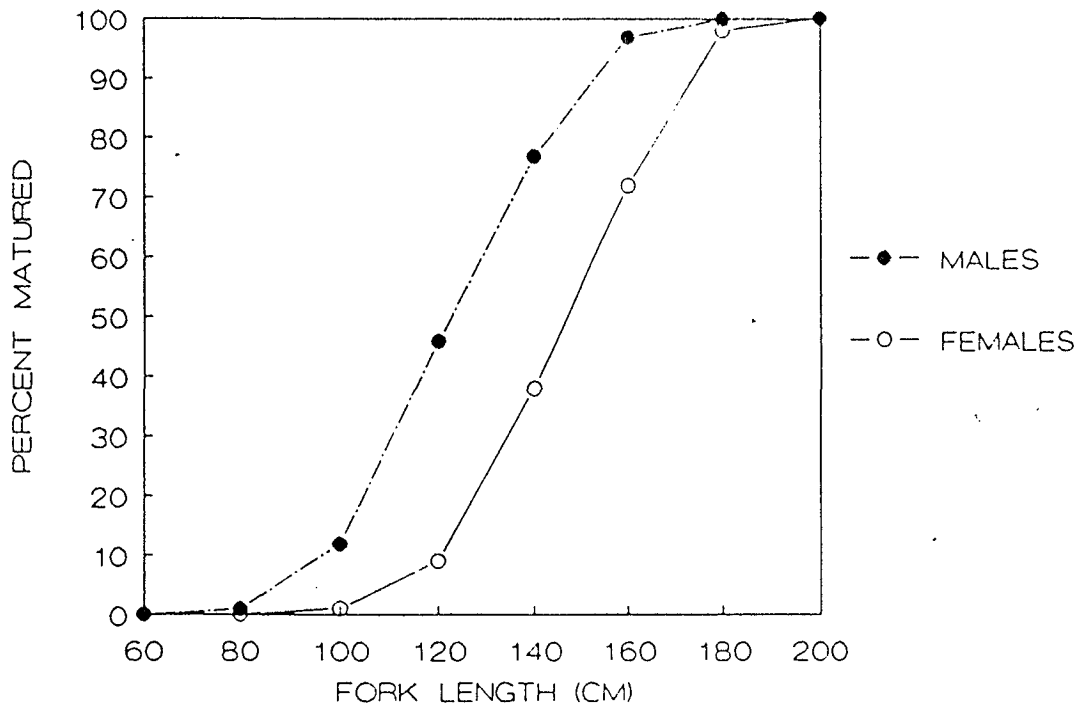


Figure 14. Percentage of sexually matured male and female sturgeon from the San Francisco Bay estuary. The average was defined as the size at which 50% of the individuals were maturing or ripe. Matured fish were represented by 321 males and 81 females.

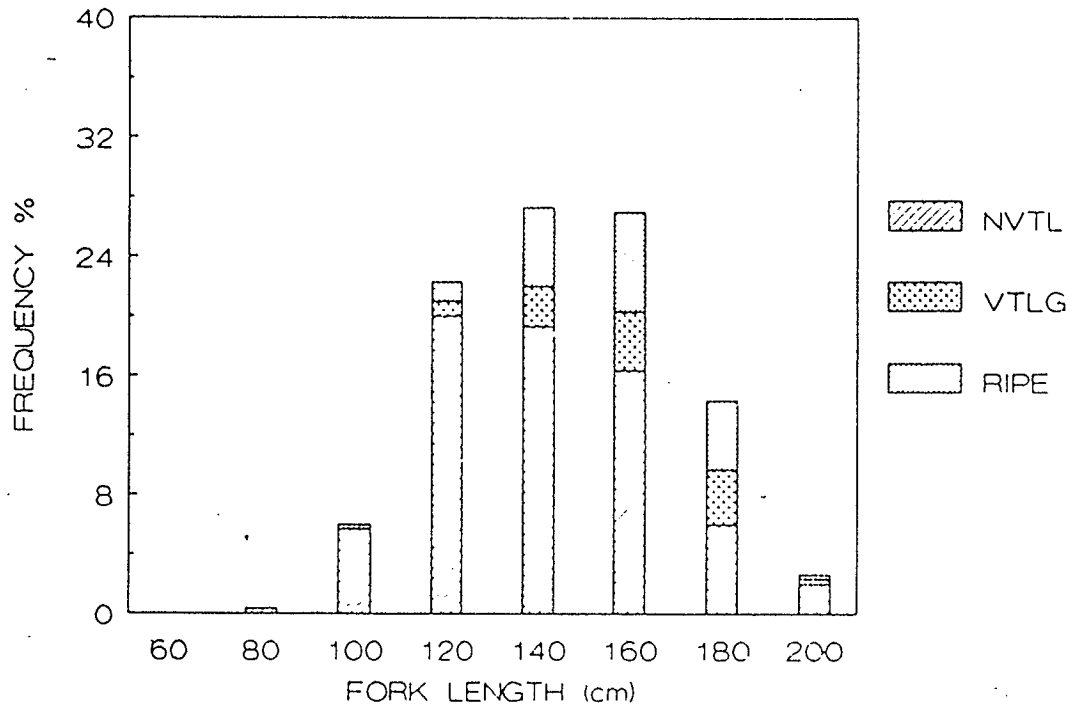


Figure 15. Histogram of different stages of ovarian development by size distribution for female sturgeon from San Francisco Bay. NVTL-non-vitellogenic (immature or resting) ovary. VTLG-vitellogenic ovary.

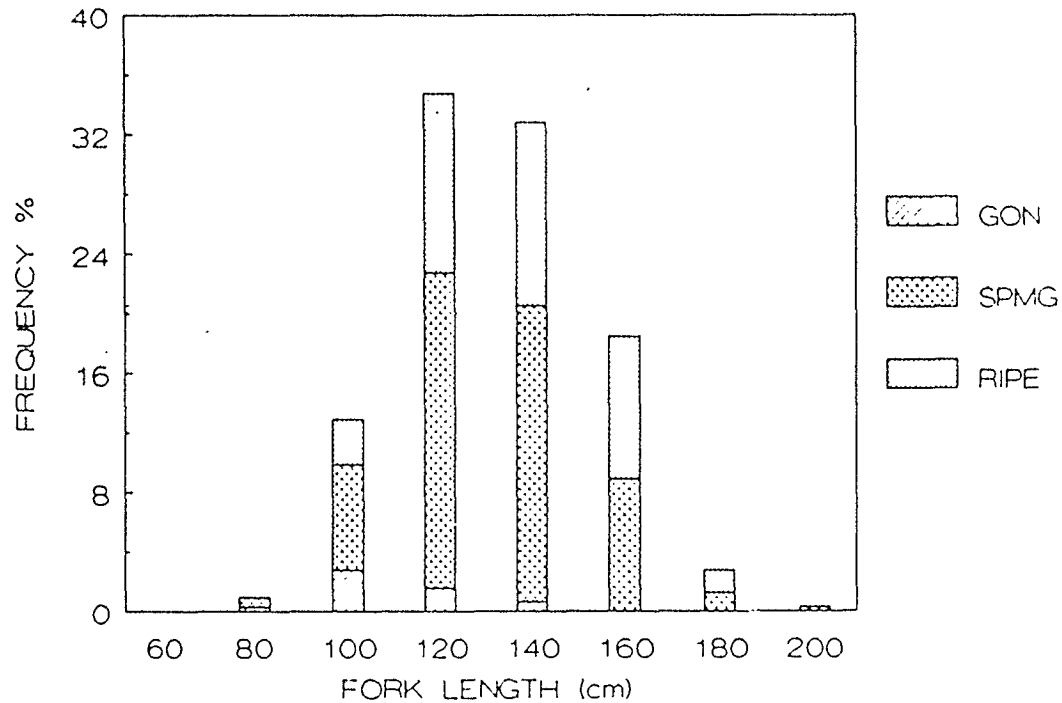


Figure 16. Histogram of different stages of testicular development by size distribution for male sturgeon from San Francisco Bay. GON-immature or resting testes. SPMG-testes in active state of spermatogenesis.

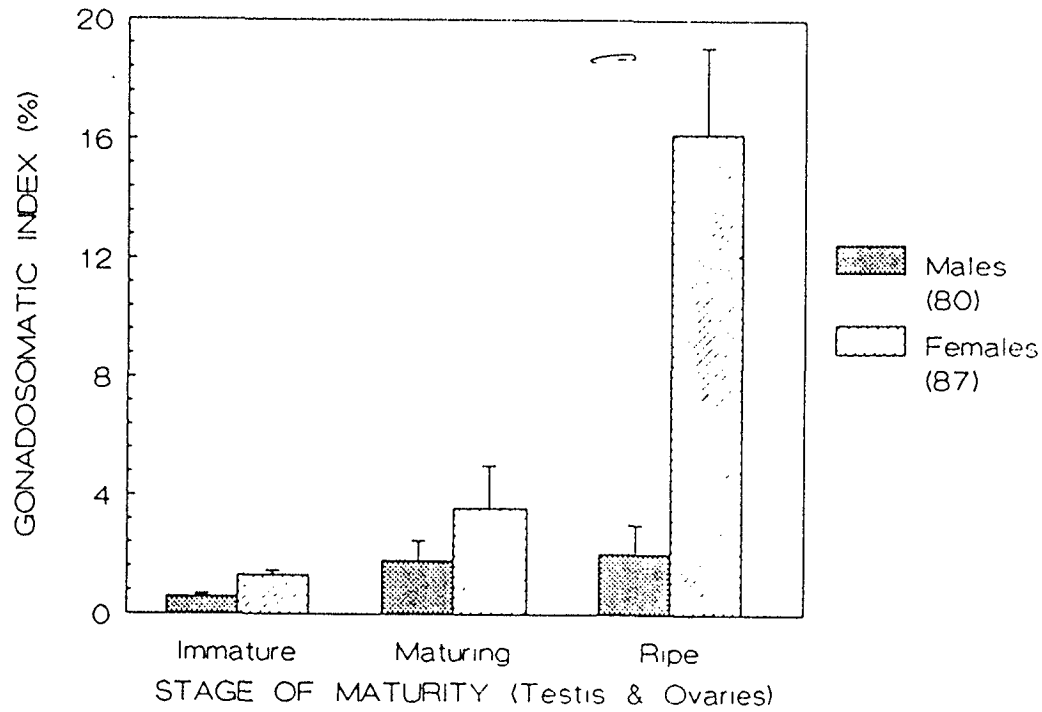


Figure 17. Mean gonadosomatic index values for male and female sturgeon from San Francisco Bay. Bars represent 95% confidence interval.

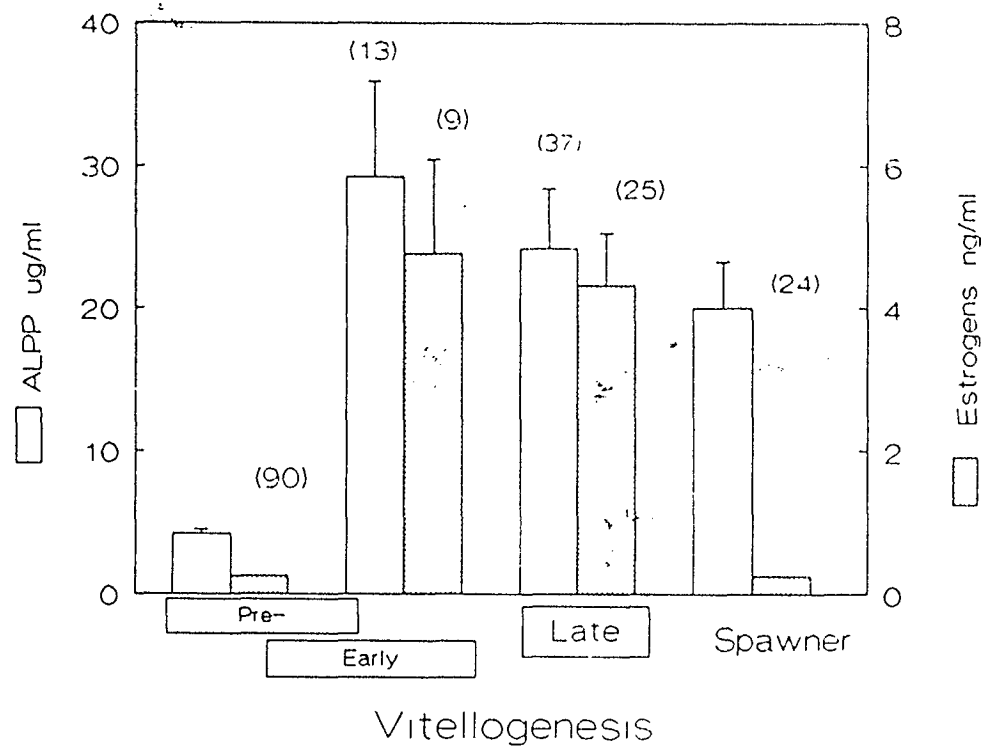


Figure 18. Plasma concentrations of total estrogens and ALPP in relation to gonadal development in white sturgeon. Means ± s.e. Numbers in parenthesis.

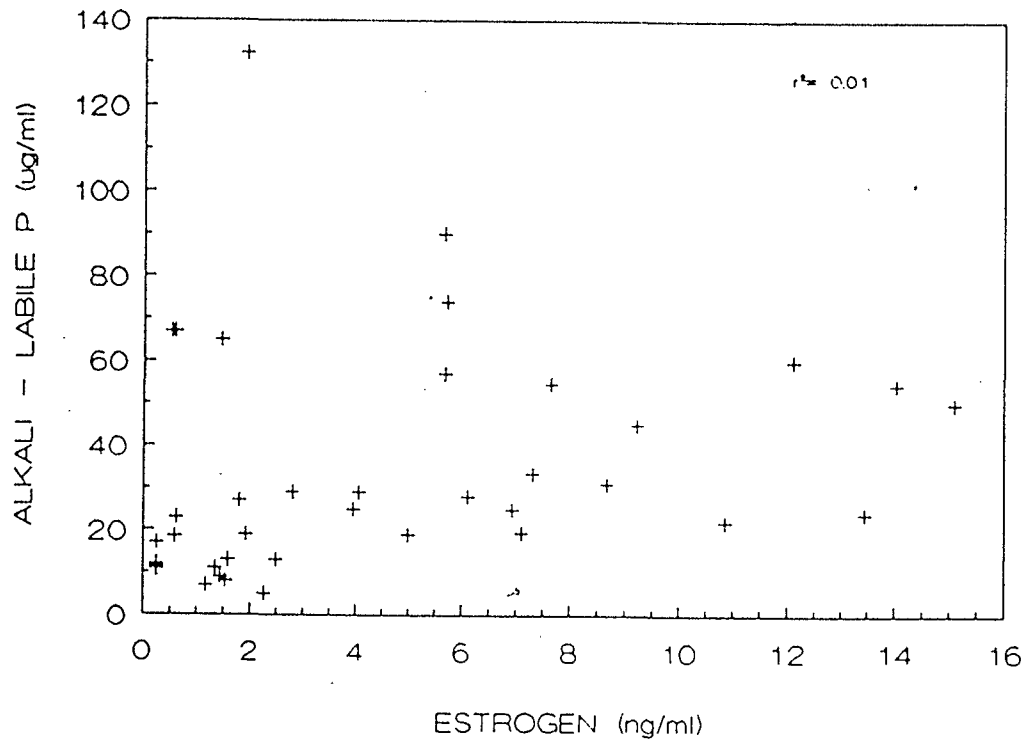


Figure 19. Scatter plot showing lack of relationship between concentrations of estrogen and ALPP in peripheral plasma of white sturgeon. N= 40.

III. GROWTH AND MATURATION OF DOMESTIC STURGEON BROODSTOCK

The population of domestic sturgeon consisted of four year classes that ranged from 4.7 to 8.5 years of age. The largest sturgeon was a female 175 cm FL long, and weighing 43.5 Kg (8.5 yrs.). The biggest male was 138.5 cm weighing 23.1 Kg (8.5 yrs.). Rearing of first generation sturgeon under captive conditions substantially influenced their growth and sexual maturation, especially in male sex.

Growth Rates and Sex ratio

The data on body weights of four captive year classes are given in Appendix 1. In general, growth was accelerated (0.7-1 Kg at age 1, 2-3 Kg at 2 yrs, 3-6 Kg at age 3 yrs, 6-7 Kg at 4 yrs, 9-10 Kg at 5 yrs, 12 Kg at 6 yrs, 15-20 Kg at 7 yrs, and 25 Kg at 8 years).

Table 4. Frequency of domestic male and female white sturgeon within age groups, with Chi-square values assuming a 1:1 sex ration

Yr. Class	Age (yrs)	Male	Female	Chi-Squares
1981	5	13	11	0.21
1982	4	42	36	0.47
1983	3	37	38	0.08

There were, however, considerable variations in size at each age, and between the year classes. Figure 20A shows the data fitted to the third order polynomial equation (R^2= 0.99). For comparison with data from wild stock (based on Kohlhorst et al. 1980), these data was fitted to a simple linear

regression equation after the transformation of body weight in the Log10 form (Fig. 20B). It is apparent that growth in captivity was greatly accelerated (approx. 1.7 times) compared to that of the wild fish. Under culture conditions, sex differentiation in white sturgeon occurred at about one and a half to two years of age, and the sex ratio did not differ significantly from 1:1 (Table 4).

Growth rates differed in different age groups and between the males and females (Table 5). Instantaneous growth rates were similarly high in both sexes until the ages 4.5-5 yrs; this coincided with sexual maturity of most males in the sampled stock. In the following years (age 5-8), males appeared to substantially decrease their growth rate whereas the females (immature at this age) continued fast growth.

Table 5. Instantaneous growth rates for male and female white sturgeon. ± S.E.M.

Age (Yrs)	3.9-4.85	4.1-4.8	4.8-5.25	5.25-5.8	5.7-6.6	6.6-7.1	7.1-7.6	7.6-8.1
Interval	0.95	0.7	0.45	0.55	0.9	0.5	0.5	0.5
Males	0.265±.054	0.332±.033	0.072±.030	0.262±.047	0.145±.023	0.086±.045	0.231±.051	0.086±.10
Females	0.387±.074	0.380±.032	0.196±.043	0.454±.130	0.211±.023	0.241±.026	0.173±.053	0.238±.05

Maturation and Reproductive Performance of Domestic Males

Spermatogenesis:

The testicular development of captive males did not differed from wild prototype (Fig. 21A & 21B). Gametocytes were grouped together in small cysts of equal maturity, and were found in the testes of adults during all seasons (Fig. 21A). During development, the cells became smaller and spermatids were recognized (Fig. 21A). Matured sperm were recognized by their characteristic elongated flagellum (Fig. 21B).

Monitoring gonadal development of several individually marked animals suggested that spermatogenesis was seasonal (Fig. 22). Histological evaluation revealed that most adult males began to mature in late Summer. Ripe fish were predominant and ready to spawn from October-November (67%) through April-May (60%). During summer months, semen production was reduced and appeared to decline with warm water temperatures. Once diminished, semen production was not resumed until about four months later.

Reproductive performance:

More than 60% of white sturgeon males raised in captivity reached sexual maturity between 2.6 and 3 years of age. Over 80% of the individuals matured each year thereafter (Table 6).

Table 6. Percentage of matured males of domestic sturgeon, by year class and age group. Sample sizes are given in parentheses.

AGE (Yrs)	YEAR OF BIRTH 1980	1981	1982	1983
THREE	N/A	N/A	53.8% (39)	85.0% (20)
FOUR	77.8% (9)	74.6% (55)	97.6% (42)	58.8% (17)
FIVE	82.8% (29)	81.3% (16)	77.8% (9)	80.0% (15)

The average size at first maturity of domestic sturgeon was 5.1 ±0.35 s.e.m. Kg/ in body weight and 84.5 ±1.88 s.e.m. cm fork length. The youngest male matured at 21 months of age, was 65.8 cm long (SL) and 1.9 kilograms in body weight. In contrast, the smallest wild caught, ripe male was 12-14 years old, 110 cm FL and 8.9 kg in weight.

Males were induced to spermiate with injections of crude pituitary extracts to evaluate semen quality. Amounts of semen produced and the concentration of sperm contained by sturgeon males varied greatly (Table 7).

Table 7. Means and S.E. of semen characteristics from ten males of 10.99 ±0.49 Kg b.w.

Semen Characteristics	Mean	S.E.
Total volume per spermiation (ml)	136.5	1.1
Sperm concentration ($x10^7$)	21.5	3.4
Motile spermatozoa (%)	87	3.2
Duration of sperm motility (min)	2:32	4.6

It was difficult to estimate the volume of semen per spermiation because milting males released their semen sporadically into the water. In 44 spermiations normal semen volume values ranged from 5 ml to 100 ml, but values of 200

ml were sometimes collected. Sperm densities varied from a few thousand to about 4 billion per ml. In fertilizing water, duration of sperm motility was approximately 2.5 min. (Fig. 23), and the percentage of motile spermatozoa was usually 60 to 100%; above 85% was considered normal.

The fertilizing capacity of domestic males semen was compared to that of wild stock, using the eggs from the same wild females. For domestic males, mean fertility was 77% compared with 75% obtained with wild stock (Table 8).

Table 8. Percentage of eggs fertilized.

MALES	DOMESTIC					WILD			
FEMALES (WILD)	1	2	3	4	5	1	2	3	4
I	96.1	89.2	97.8	69.6	92.4	94.9	95.4	14.2	N/A
II	92.8	40.6	70.3	74.9	70.8	79.4	70.4	49.2	80.9
III	66.9	88.1	72.8	86.4	87.5	37.8	69.0	76.3	86.6
IV	91.2	N/A	83.8	81.9	90.9	89.2	94.3	94.9	93.3
V	73.5	62.6	66.9	64.7	61.5	68.5	67.8	70.5	78.4

ANOVA DATA

Source	df	MS	
Strain	1	108.83	N.S.
Female	4	653.68	N.S.
Male (strain)	7	269.60	N.S.
Residuals	30	271.77	

$Y(ijk) = \mu + Si + Fj + M(S)ik + Eijk$

A 6% of abnormal fertilized ova was observed in the captive stock semen treatment, but the difference was attributed to polyspermy. Table 9 shows the fertilization of eggs achieved with sperm from domestic and wild stock males. The evaluation of semen on the basis of sperm density, and spermatozoa motility also did not show significant differences between the captive and wild stocks (Table 9).

Table 9. Semen characteristics of wild and domestic stocks of white sturgeon. Mean ± s.e.m.

Semen Characteristics	Wild	Domestic	Sign.
Sperm motility (%)	74 ± 3.0	77 ±3.0	N.S.
Duration of motility (min)	2:40 ±10.6	2:35 ±6.8	N.S.
Sperm density ($x10^7$/ml)	31.39 ± 9.9	18.79 ±2.4	N.S.

Maturation of Domestic Females

In spite of high growth rates, no sexual maturity was observed in any domestic females until the age 8 yrs, although there were some changes in differentiation of the ovarian follicles in age groups 7 and 8. Therefore, this section presents preliminary data.

Ovarian histology:

When sex differentiation occurred (age 1.5-2 years) oocytes were already present in the ovary and arrested in the first meiotic division. During the first year of development, the ovaries of domestic females were characterized by the presence of oogonial follicles in transition to primary oocytes (Fig. 24A). Once the primary oocytes were formed, gonial cells entered a period of arrest. The duration of this process remains unknown. These oocytes were in early growth phase, with basophilic cytoplasm and poorly differentiated follicular envelope. The follicular envelope consisted of undifferentiated granulosa cells and lack a zona radiata.

Three fish of age groups 7 and 8 exhibited differentiation of granulosa layer and zona radiata, and one fish (age 7.5, Wb= 16.2 Kg) had recently initiated vitellogenesis (Fig. 24B). The lack of precise physiological information for early stages of vitellogenesis, made it difficult to identify observed progress towards sexual maturation. In all females examined levels of alpp fluctuated between 1.0 to 11.75 μg/ml and, except for the

vitellogenic female (1.3 ng/ml) plasma estrogens were below detectable levels (i.e. 250 μg/ml). Currently raised colonies of domestic females are now approaching the size distribution of the mature females (Fig. 25). Further observation will determine whether the age or body size are major determinants of female sexual maturation, and how they interact. Important factors to take into consideration are unknown effects of the culture environment and genetic variability in sturgeon stocks.

Stimulation of vitellogenin synthesis:

Administration of estrogen (17B-estradiol) induced a marked increase in plasma phosphoprotein (i.e. alpp) and total calcium levels in male and female Acipenser transmontanus (Table 10).

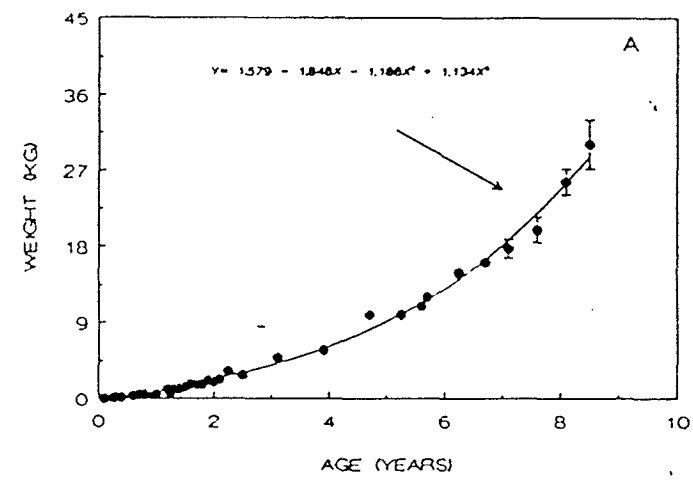
Table 10. White sturgeon concentration of plasma Alkali-labile phosphorous (ALPP) and calcium. Before and after 28 day implantation.

Group	ALPP μg/ml ± S.E.M.	Ca μg/ml ±S.E.M
Normal males	4.4 ± 1.3	83.9 ± 2.5
Estrogen treated males	296.2 ±28.9	514.8 ±39.6
Non-vitellogenic females	4.3 ± 0.5	80.7 ± 0.7
Estrogen treated females	343.8 ±30.3	730.8 ±61.7

We also observed an ALPP-estrogen dose response when sturgeon were treated with different dosages of 17β-estradiol. Figure 26 shows the time course of accumulation of alpp in sturgeon plasma after treatment with 17β-estradiol. A marked increased in the amount of alpp was

seen at day fourteen and thereafter at an increasing rate per day. At about 35 days, plasma levels of alpp peaked around 400 ug/ml and remained elevated thereafter. The route of administration and amount of drug administered also appeared to be influential in the drug response. Intraperitoneal implants were more potent than intramuscular implants but no significant difference ($P > 0.01$) were observed between the two higher dosages.

We also observed a correlation between the total calcium and protein phosphorous according to the relationships: ALPP= 0.46 x Calcium - 32.73; total Ca= 75.28 + 2.06 x ALPP. Where alpp and calcium are expressed in μg/ml of plasma. Since in normal males and non-vitellogenic females values of ALPP and Ca were in the same low range, these relationships probably reflect the induction of hepatic vitellogenin synthesis, and be considered useful indicators of reproductive activity. The repeated experiments with E2 implanted sturgeon females confirmed this finding that estradiol stimulates secretion of liver vitellogenin (Doroshov and Moberg pers. comm.), however, it was found that E2 does not induce ovarian differentiation and does not stimulate vitellogenin uptake by the oocyte.



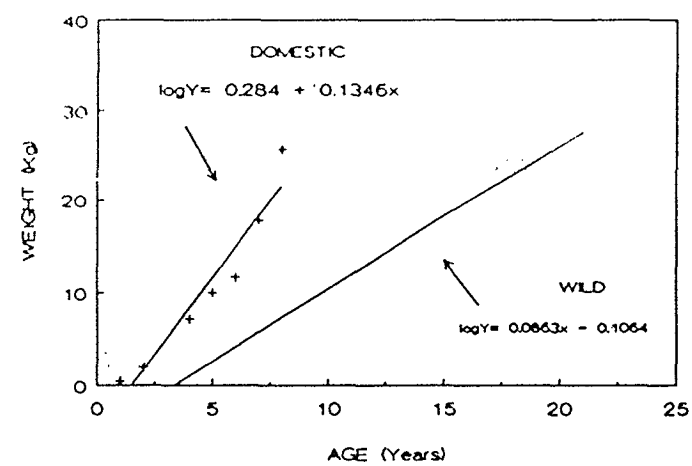


Figure 20. (A) Growth curve for combined year classes of domestic sturgeon. Curve was fitted using third order polynomial regression (r^2= 0.99). (B) Growth regression of domestic and wild stock white sturgeon.

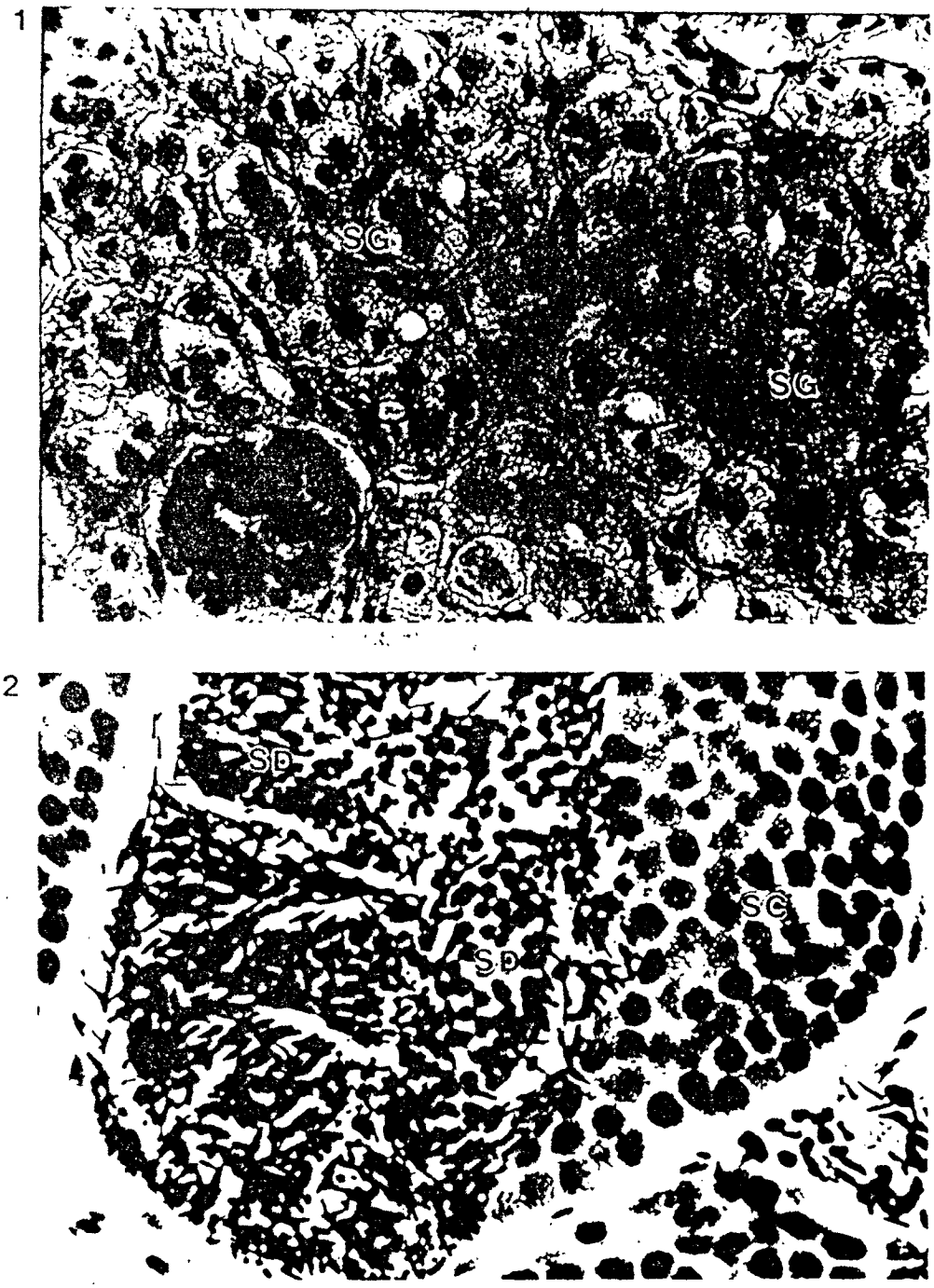


Figure 21A. Cross section through testis tubules of domestic sturgeon. 1) Clusters of spermatogenic cells (SG). 2) Differentiation and proliferation of primary and secondary spermatocytes (SC); spermatids (SD). H & E. X630.

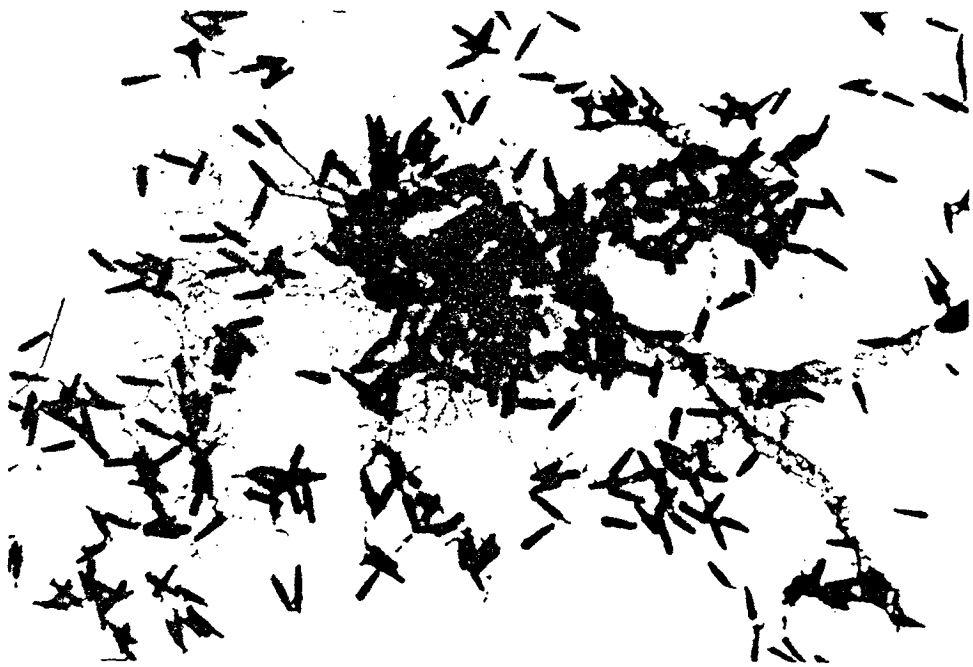


Figure 21B. Spermatozoa in lumen of matured testis tubules of domestic white sturgeon. H & E. X630.

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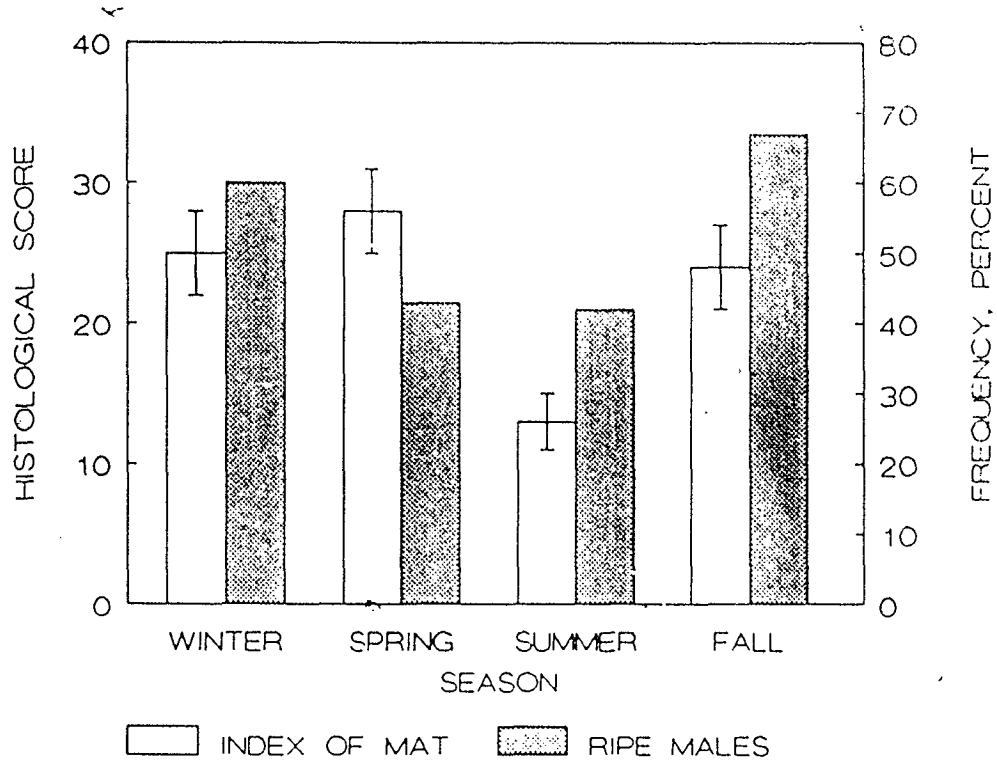

Figure 22. Frequency and histological score of maturing captive males during different seasons. Bars represent standard error of the mean. N= 54.

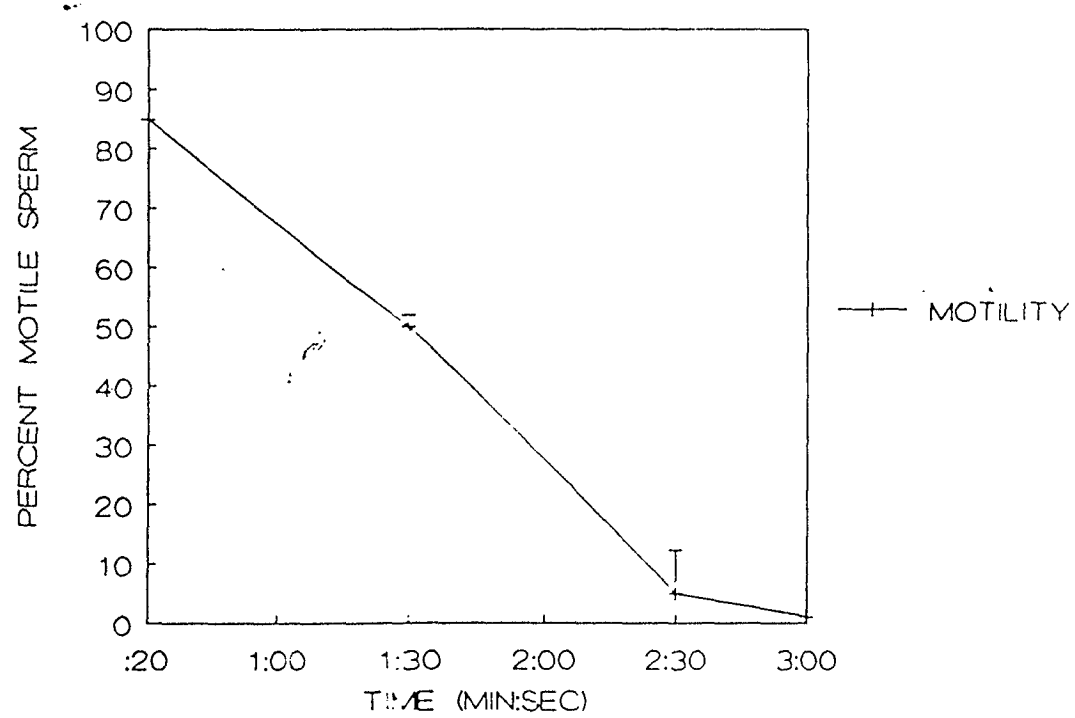

Figure 23. Duration of motility of sturgeon spermatozoa. Bars represent s.e.m.

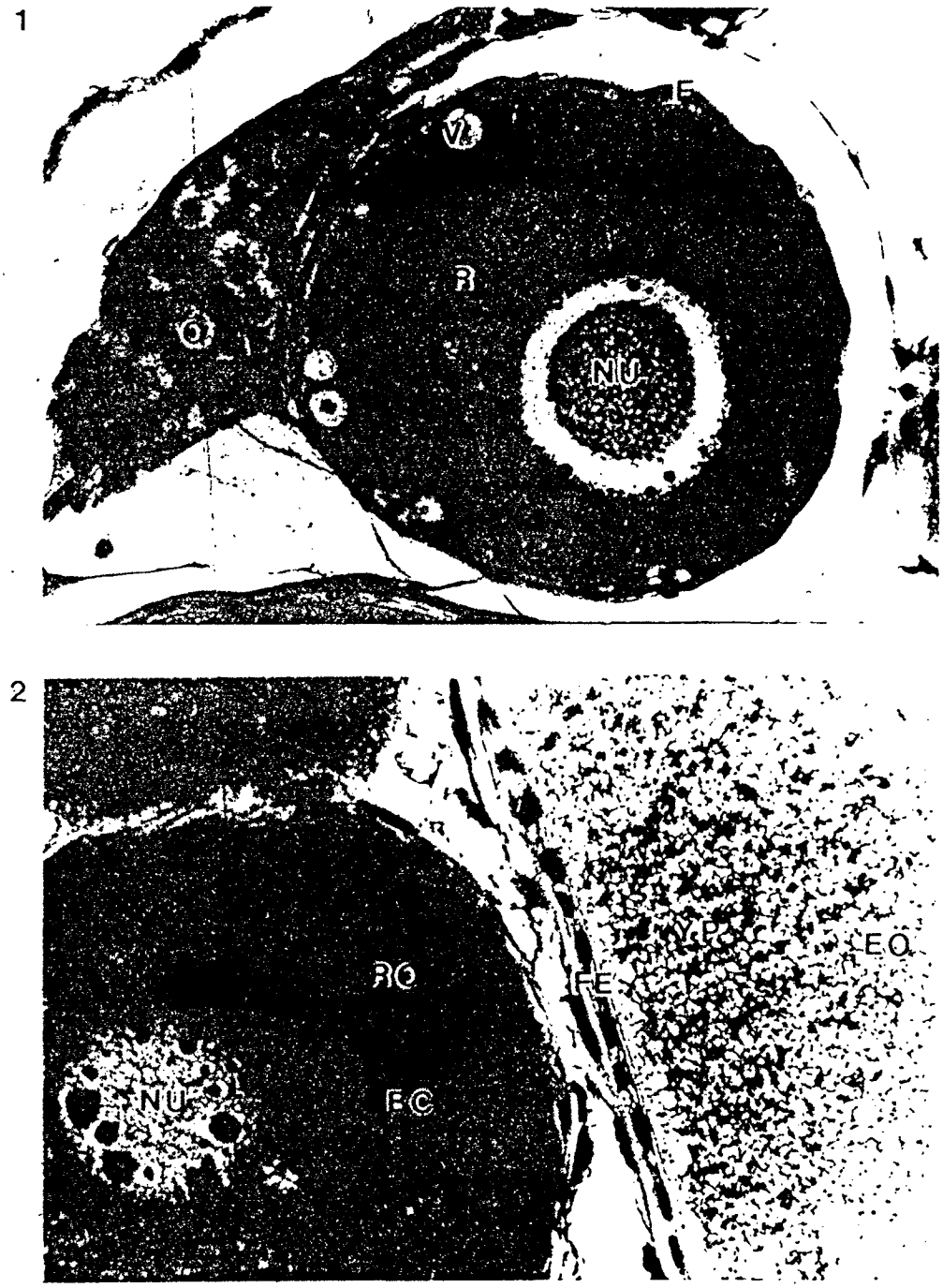

Figure 24. Oocytes in captive broodstock white sturgeon. 1) Early (O) and resting (R) follicles; nucleus (NU); vacuoles (V); undifferentiated follicular envelope (F). 2) Early vitellogenic oocyte in maturing female. Differentiating follicular envelope (FE); eosinophilic cytoplasm (EO); yolk platelets (YP); resting oocyte (RO); basophilic cytoplasm (BC); nucleus (NU). H & E. X400.

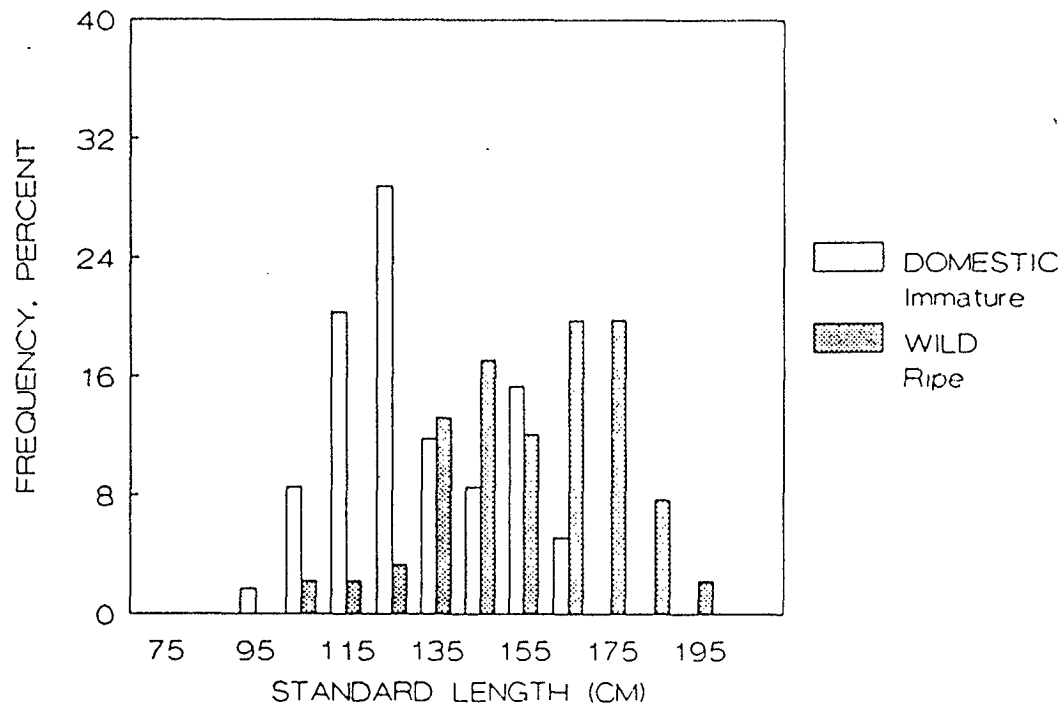

Figure 25. Body size in FL of matured wild and immature domestic female sturgeon stocks.

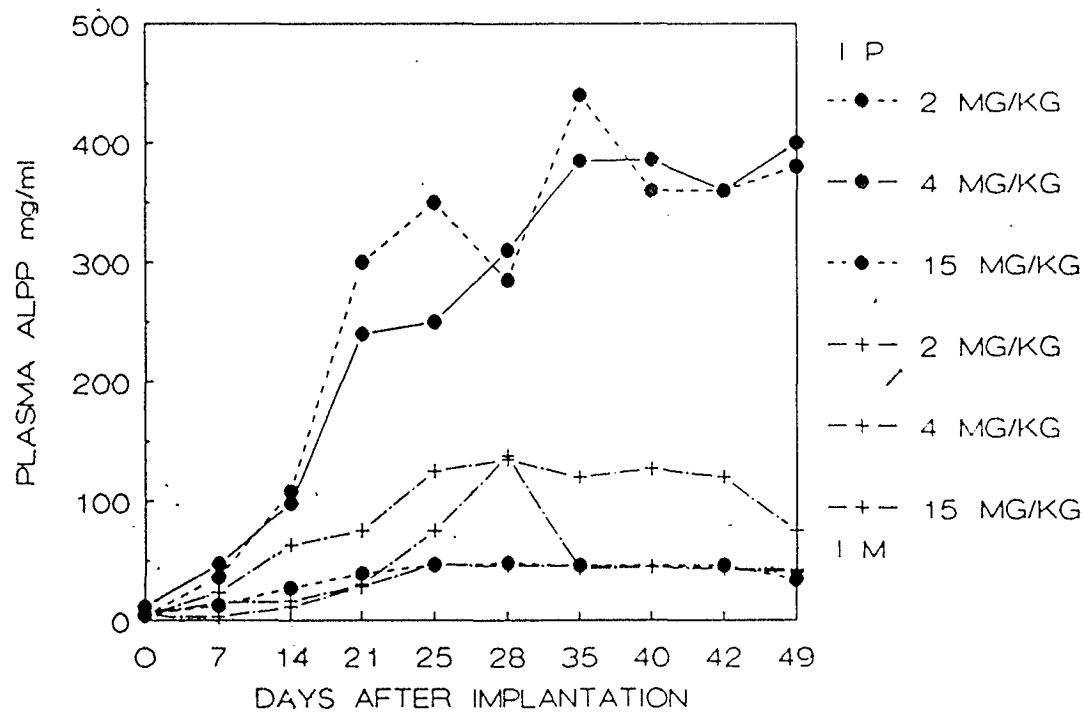

Figure 26. Accumulation of ALPP in sturgeon plasma after treatment with 2, 4, and 15 mg/Kg b.w. of 17β-estradiol.

DISCUSSION

Observations on wild and domestic broodstocks indicate that white sturgeon have an unusually long and complex process of gonadal development. Since our data do not cover all the stages and events involved in this development in white sturgeon, we can offer some general description of gametogenesis that combine our observations in white sturgeon and available information concerning gametogenesis in other sturgeon species and modern teleost fish.

I. Gametogenesis and the Reproductive Cycle in Wild Stocks:

During a reproductive cycle, the ovaries and testes of sturgeon undergo marked changes in metabolic activity, morphology, and tissue growth. Associated with these changes is the secretory differentiation of the pituitary-gonadal, endocrine axis (Barannikova et al. 1985; Barannikova & Fadeeva 1982), accompanied by the hypertrophy of the vascular system, liver tissue and metabolism, and changes in the somatic body tissues (Dorosheva 1983; Krivobok and Tarkovskaya 1970). Although the gonadal development is a continuous process, we view the gametogenic cycle in sturgeon t[redacted]st of three, functionally discrete, developmen[redacted]es.

Phase [redacted]sts of immature or resting (e.g. intervals between th[redacted]cles) animals. The gonads are thin chords containin[redacted]ntiated follicles in females, and undifferen[redacted]ysts with only spermatogonial cells

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undergoing mitotic division in males. During this phase of development, the secretion and mechanism of action of reproductive hormones are unknown. In general, secretion of hormones is considered low and undetectable (Barannikova et al. 1985 & 1982). During this phase there is a rapid growth in body weight and deposition of gonadal adipose tissue. Evidently in females, the differentiation of the primordial follicle occurs at the beginning of the individual's reproductive cycle and is absent during repeated reproductions. Similar to other mammals, elasmobranchs, birds, and some fishes, (Van Tienhoven 1983) adult sturgeon do not exhibit proliferation of oogonia. Our histological observations provide no evidence of mitosis occurring soon after sex differentiation or puberty. Resting oocytes, appear to persist throughout the reproductive life and serve as a source of oocyte recruitment during each vitellogenesis. Similar observations have recently been made with a wild stock on Russian sturgeon, Acipenser guldenstadti, in the Sea of Azov (Kornienko et al. 1988). However, no experiments to support this view were attempted. Prominent structures during this developmental period are the peripheral vacuoles during stage 2 of oogenesis (Fig. 5). These structures are also analogous in developmental time to "primordial cortical alveoli" in teleost fish described by Sellman et al. (1987, 1986). Their carbohydrate content in sturgeon oocytes remains unclear, and the time involved in their appearance and disappearance deserve a

closer examination towards their role. Perhaps their function is associated with energy requirements for the prolonged period of autosynthetic oocyte growth during stage 2. The follicular structures involved in the secretion of the female steroids and transport of vitellogenin, such as differentiated granulosa cell layer, zona radiata, and vascular bed of thecal layer, are absent during the phase 1.

The phase 2 is the active period of gonadal development that includes pre- vitellogenic and vitellogenic oocytes in females, and spermatocytes in various stages of meiosis in males. During this phase, gonads rapidly increase in weight due to proliferation and differentiation of germ cells in male (spermatogenesis) and the cell growth and deposition of ydlk in the ovarian follicle of female (vitellogenesis). Female liver is also hypertrophied and secretes yolk precursor, vitellogenin. Pituitary gonadotropin secretion is detectable, and endocrine tissue of the gonads secrete sex steroids, testosterone and estrogen (Barannikova et al. 1982). At the end of this phase the somatic growth ceases, and lipid reserves are depleted (Trusov 1975). The mechanism of sturgeon vitellogenesis appears similar to other primitive fish (e.g. elasmobranchs, Craik 1978a,b and hagfish, Yu et al. 1981) and modern teleosts (e.g. cyprinids, Khoo 1979, grouper, Tam et al. 1983 and salmonids, Crim and Idler 1978). Liver secretion of yolk precursor is initiated by the ovarian estrogens, and possibly, potentiated by cortisol. Uptake of circulating

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vitellogenin by oocytes is probably similar to other fishes and anurans, accomplished by micropinocytosis and appears under control of pituitary gonadotropin (Wallace et al. 1987; Wallace 1985; Campbell 1978). Values for plasma estradiol and ALPP obtained in the sturgeon were similar as those reported in other fishes (Dodd and Sumpter 1984). In the present study, however, although concentrations of both metabolites exhibit similar trends during the ovarian cycle, we found no correlation between the estrogen and ALPP in a group of individual fish undergoing vitellogenesis. Even though the estrogen stimulates hepatic protein synthesis, plasma levels of vitellogenin may depend on rates of synthesis, degradation, and uptake. Additionally, vitellogenin secretion can be initiated by estrogen stimulation but thereafter plasma levels of vitellogenin may not be strictly under estrogen control. Our data also indicates that oocytes of sturgeon first grow to a point immediately prior to vitellogenesis, then temporarily reside in arrested stage. This growth into vitellogenesis can be quite prolonged in time. However, we can now identify the beginning of vitellogenesis by differentiation and proliferation of the granulosa cell layer and appearance of the zona radiata. Knowledge of this transition period is extremely important because at this stage the oocyte becomes competent to yolk accumulation and the immature animal enters pubertal phase.

Phase 3 consists of mature or ripe females with

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pigmented and polarized eggs, and males containing mature spermatozoa. During this time the liver undergoes atrophy, and gonadal tissue is hydrated and ducts hypertrophied. The ovarian follicle undergoes maturation (first meiotic division) and ovulation. The spermatozoa are freed in testicular lumen. The pituitary gland is hypertrophied, and a surge in secretion of gonadotropin and steroid hormones stimulate final gonadal maturation and spawning behavior (progesterone and 11-ketotestosterone). Like in many other vertebrates (Masui and Clarke 1979), the final phases of oogenesis in sturgeon, such as oocyte maturation and chromosomal reduction are not completed until the egg is ovulated and fertilized (Raikova 1976). These changes can be induced in vitro by stimulation with steroid hormones (Lutes 1984) and used as a valuable indicator of ovulatory response to spawning induction (Lutes et al. 1987). In teleost fish, oocyte maturation is also preceded by a rapid and transient increase in plasma levels of gonadotropin hormone (Scott 1987; Crim et al. 1975). Barannikova et al. (1982) demonstrated that there is a preovulatory surge of gonadotropin hormone prior to oocyte maturation and ovulation in the female of stellate sturgeon, A. stellatus. Ivanova (1954) and Detlaf and Davydova (1979) also suggested the involvement of thyroid hormones during oocyte maturation. Our observations suggest similar mechanisms for these processes in the white sturgeon. Oocyte maturation (in vitro) and ovulation (in vivo) can be induced by

administration of GtH extracts and GnRH analogs. Ovulatory response and egg quality appears also to be improved after administration of T3 hormone (unpublished observations).

Atresia is another important phenomenon of the ovarian cycle in fishes. However, only a few studies of this process have been made to date (Byskov 1978; Hunter & Macewicz 1980, and Saidapur 1978). In white sturgeon, atresia occurred throughout the ovarian cycle but pre- ovulatory and anovulatory atretic follicles were most prominent. It appears that the number of eggs which become atretic are primarily independent of environmental variation. In other fish species, these numbers can be altered by various environmental factors, such as nutrition, food deprivation and handling stress (de Vlaming 1977a, 1972). Furthermore, these effects could occur within a very short time span. The control of the number of developing follicles and that of mature follicles which ovulate could be a very interesting and important area of research. In the sturgeon literature, I found no evidence of endocrine function associated with atretic follicles. White sturgeon atretic follicles were absorbed quickly and did not formed corpus luteum-like bodies; even though anovular egg pigments remained for prolonged periods of time.

The adipose tissue adjacent to the sturgeon gonads appears also as an essential component of gonadal development. Its relationship may be functional as to supply the gonad with energy and various metabolic substrates

during the prolonged period of development.

Based on the proportions of different stages of development, in wild stock of San Francisco Bay, we conclude that the duration of one reproductive cycle in white sturgeon is approximately 4 to 5 years. Stage 1 (resting) will be recruited to breeding population after at least two or more years. Stage 2 will breed the following year, and stage 3 is the current year broodstock that would enter the river in the Fall and will spawn next spring. Since sturgeon females lose more body calories at spawning than any other fish (Krivobok and Tarkovskaya 1970), this timetable of the ovarian cycle in iteroparous sturgeon allows recovery and growth of fish needed for each new wave of vitellogenesis. We assume that the reproductive potential of sturgeon is very low, since only small proportion of adult fish are recruited for annual spawning. Similarly in other sturgeons, according to Trusov (1975), quiescent ovarian stages have a long duration (2 to 5 years between two cycles), while the vitellogenesis occurs within the period of 1-2 years. Recent analysis of sexual maturation in the wild stock of Russian sturgeon indicates striking similarity with our data on white sturgeon (Kornienko et al. 1988). 72% of sampled adult size fish, captured in the Azov Sea, were in stage 2 of the gonadal development, with the remaining fish approximately equally distributed between the stages 3 and 4. In males spermatogenesis was a continuous process which perhaps accommodates to a protracted spawning season by females. The

1:1 ratio of females to males in the immature stock and 1:2 in the spawning stock, perhaps too reflect the faster recruitment of males.

II. A model for the initiation of sexual maturity:

White sturgeon attain sexual maturity at an advanced age and large body size (this study and those of Semakula and Larkin 1968). Likewise, age at first sexual maturity in other acipenserid species ranges 8-15 years in males and 10-25 years in females (Berg 1948; Kozin 1964). These ranges, however, vary considerably and perhaps in part, associated with variability in growth rates (Semakula and Larkin 1968; Chugunov and Chugonova 1964). Persov (1975) attributed late sexual differentiation in sturgeon to its slow growth. Under the hatchery conditions, however, growth of sturgeon is accelerated, with maturation of males occurring regularly and in early age. In captive females, sexual maturity and ovarian development appear to be correlated with age and body size. We know of three ongoing attempts (France, Japan and the Soviet Union) to establish domestic broodstock of different sturgeon species (Williot and Brun 1982 working with A. baeri; Kijima and Maruyama 1985; Burtzev 1967 working with a hybrid of A. ruthenus male x Huso huso female). These attempts revealed acceleration of sexual maturity in females to age range 7-10 years, and its relationship to growth and feeding. All were fed artificial diets and received natural food supplements. Based on these observations, feeding and growth conditions should be

determining factors in acipenserid reproductions. The above studies, however, provide little information on the gametogenesis and do not allow for conclusions on the causative factors and mechanisms involved in sexual maturation on captive sturgeon species. We thus provide a hypothesis to be tested in future experiments.

We propose that the age of first sexual maturity in sturgeon is dependent on metabolic body size and body nutrient composition, and could be modified by growth, nutrition, and environmental factors, within the wide range of the genetic time clock (Fig. 27). This assumption is based on two observations made during our work with wild and captive broodstocks. First, captive males raised in tanks matured invariably at early age (3-5 years) compared with wild caught males (10-15 years). Second, the lack of maturity in at least two age groups of domestic females at UCD facilities that exceed 20 Kg. During the history of sturgeon spawning at UCD and commercial farms in Northern California, there were a substantial number of gravid females with body weights below 20 Kg, caught in the Sacramento River (S.I. Doroshov and K. Beer pers. comm.).

It appears, therefore, there is a close inter-dependence between gonadotropic function and extraneous factors. In this case for example, the nutritional status of the organism can accelerate or delay the attainment of sexual maturity (e.g. Foster et al. 1986 & 1985; Bronson and Rissman 1986; Levasseur 1977). The concept

is schematically shown on figure 27.

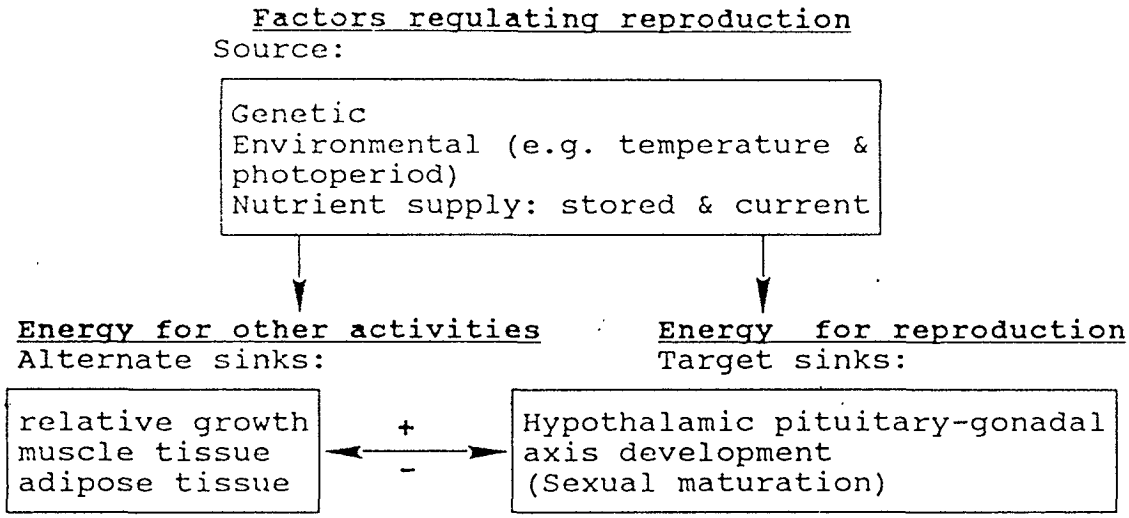

Figure 27. Proposed conceptual approach for initiation of sexual maturity in sturgeon.

With regards to the lack of sexual maturation in domestic sturgeon females over 20 Kg, the major cause is most likely the result of their altered metabolic state, for example, of inadequate nutrition. A process or substance will promote maturation if it activates the target sink or inhibits the alternate sink. The corollary proposition is that any process or substance that inhibits diversion of energy to non-reproductive activities would appear as a promoter of sexual maturation. Also any process or substance that inhibits maturation may promote activity of competing sinks, thereby reducing assimilated (e.g. nutrient) supply to the target tissues. Sexual maturation will also ensue if there is an manipulation of the source factors regulating reproduction, e.g. in the hatchery through genetic selection

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or environmental cues.

Associated with these interactions is the question, whether or not environmental manipulations (e.g. hormonal implantations) alter the hypothalamic axis, and in effect increase or decrease source strength. This question was addressed recently by our group (Doroshov and Moberg pers. comm). The preliminary results from the first experimental study were negative (Moberg et al. submitted). In other fishes, most attempts to "induce" vitellogenesis (hence sexual maturation) in immature females by the administration of reproductive hormcnes were unsuccessful (Crim 1984; Crim et al. 1983; Aida 1983; Lee et al. 1986; Magri et al. 1985); with the exception of Dufour et al. (1984), who reported induction of vitellogenesis in immature eel by combined treatment with estrogen, GnRH and pimozide (an anti-dopaminergic substance). Therefore, we can assume that interactions between the alternate and target sinks (Fig. 27) in sturgeon females, and in fish in general, are very powerful in determining the initiation of reproductive cycles, whereas in males such interactions can be easily overcome by invasive hormonal therapy technique (Lee et al. 1986; Weil and Crim 1983; Weil et al. 1978; Yamazaki 1972; Clemmens et al. 1966; Eversole 1941). An example of such interaction in sturgeon females is the dramatic increase of muscle fat content (from approximately 3 to 20%, per gram dry matter) during the initiation of vitellogenesis reported for three wild sturgeon stocks in different geographic

areas: Acipenser stellatus and A. guldenstadti from the Caspian Sea (Krivobok & Tarkovskaya 1970); A. guldenstadti from the Azov Sea (Kornienko et al. 1988); and Huso dauricus from the Amur River (Krykhtin 1987 & 1978).

Other environmental factors, such as temperature and photoperiod have been suggested to be important parameters for maturation in fishes (de Vlaming 1972b). In the sturgeon, spermatogenesis appears to be affected by temperature. It is not unlikely that oogenesis can also be altered, and in this case a reduction in water temperatures will be important in stimulating a particular stage of development (e.g. vitellogenesis). This proposition has also been suggested for the channel catfish and other temperate species (de Vlaming pers. comm.). However, we should note that "stimulation" of vitellogenesis by decreased water temperature may be actually activation of gonadotropin (e.g. FSH-like) secretion leading to complete differentiation of granulosa layer and initiation of the ovarian estrogen secretion; whereas the vitellogenesis per se is accelerated by increasing water temperature (de Vlaming 1974). Our preliminary histological observations indicate that pre-vitellogenic differentiation of the ovarian follicle in sturgeon occurs during the cold season whereas major yolk deposition and oocyte growth appear to occur during the summer months.

Schreibman and Kallman (1977) demonstrated the influence of genetic factors in the initiation of sexual

maturity in the platyfish. In species where sexual maturation is easily attained, and generation intervals are short, this is perhaps the most practical approach in manipulating maturation. In sturgeon, however, the time involved will make, at least initially, this approach almost prohibitive. When broodstock husbandry and sturgeon domestic egg production are established, there will be needs to investigate relationships between the growth, size, age, and the initiation of sexual maturity. Several lines of evidence suggest that growth causes changes in physical and chemical constants that alter the central nervous system relations with the neuro-secretory functions of the hypothalamus (Levasseur 1977). There is a close inter-dependence between initiation of gonadotropic function and growth (Foster et al. 1986 & 1985). Puberty is closely related to metabolic state, and changes in plasma concentrations of insulin, glucose, free amino acids, and fatty acids (Steiner et al. 1983). Therefore, in order for puberty to ensue, some type of growth-related endogenous cues must be used by the organism to recognize the time for the initiation of sexual maturity (Steiner et al. 1983; Thorpe 1986; Policansky 1983; Pauly 1984). The potential for interaction of growth substances (metabolic cues) may activate target sinks by increasing the hypothalamic-pituitary activity.

There is no simple explanation for the complex nature of gametogenesis and the initiation of sexual maturation in

sturgeon species. I have recorded, however, the major morphological events of gametogenesis in wild caught and domestically raised white sturgeon, and attempted to correlate them with available physiological data. I hope they can serve as a framework for future studies to obtain a more complete view of sturgeon gametogenesis, and accelerate the age of sexual maturity in sturgeon culture.

A long time ago, Monastyrsky (1949) noted that complex age/size structure of sturgeon spawning populations, and the longevity of reproductive processes in these species require a cardinally different approach to fishery management. Our data on gametogenesis in the white sturgeon stock of San Francisco Bay provide the first direct evidence for potential sensitivity of sturgeon to their commercial exploitation (by either commercial or sport fishery), aggravated by the reduction of spawning grounds due to river damming and environmental pollution. Two major considerations could be important for future management of white sturgeon stocks: (1) that only a small proportion of the total adult stock participates in annual spawning; (2) that the spawning population consists of complex mosaics of age/size classes, due to the longevity of female life and the different intervals between reproductive cycles in the two sexes.

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APPENDIX

Body weights in Kg (mean ± s.e.) by year class for domestic white sturgeon. Age in years; numbers in parenthesis.

AGE	YEAR CLASS			
YEARS	1980	1981	1982	1983
1.0	0.35±29(61)		0.66±15.8(130)	
1.2				1.0±15.1(159)
1.25		0.72±.03(107)		
1.3	1.1±.03(111)		1.15±.04(140)	
1.4		1.21±.06(25)		
1.5	1.39±.04(127)			
1.6		1.72±123(25)		
1.7	1.68±.05(103)			
1.8		1.90±.12(25)	1.58±.1(35)	
1.9		2.16±.57(52)		
2.0		2.01±.1(35)		
2.1		2.33±.17(52)		
2.25	3.34±.09(60)			
2.5		2.80±.1(93)		
3.1	6.2±.25(29)	3.58±.12(93)		
3.9	9.0±.4(35)			7.54±.22(32)
4.1			7.11±.2(89)	
4.2				6.5±.19(43)
4.7	11.56±.57(49)			8.31±.25(35)
4.8			9.04±.25(87)	
4.85		8.9±.52(35)		
5.2				10.03±.37(44)
5.25			9.95±.38(56)	
5.6		11.01±.65(34)		
5.7	13.73±.72(45)			10.6±.41(34)
5.8			11.7±.63(50)	
6.1		11.7±.9(25)		
6.25			15.0±.78(28)	
6.6	18.0±1.82(36)	12.6±1.5(24)		
6.7			16.2±.68(37)	
7.1	21.1±1.1(28)	14.4±1.1(22)		
7.6	23.3±1.6(23)	16.7±1.4(17)		
8.1	25.8±1.6(23)			
8.5	30.1±1.5(18)			